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(71) Applicant *(for all designated States except US)*: CARGILL, INCORPORATED [US/US]; 15407 West McGinty Road, Wayzata, MN 55441-5624 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: KODALI, Dharma [IN/US]; 710 Olive Lane North, Plymouth, MN 55447 (US). FAN, Zhegong [CN/US]; 4429 Monte Carlo Place, Fort Collins, CO 80525 (US). DeBONTE, Lorin, R. [US/US]; 3706 Rochdale Drive, Fort Collins, CO 80525 (US).

(74) Agents: MCCORMICK GRAHAM, Monica et al.; Fish & Richardson P.C., P.A., Suite 3300, 60 South Sixth Street, Minneapolis, MN 55402 (US).

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(54) Title: PLANTS, SEEDS AND OILS HAVING AN ELEVATED TOTAL MONOUNSATURATED FATTY ACID CONTENT

(57) Abstract

Plants, seeds and oils having a total long-chain monounsaturated content of at least about 82 % and an erucic acid content of at least about 15 % are described. Methods for producing plants having the profiled fatty acid content are also described.

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Plants, Seeds and Oils Having an Elevated Total Monounsaturated Fatty Acid Content

Technical Field

This invention relates to fatty acid desaturases and nucleic acids encoding desaturase proteins. More particularly, the invention relates to nucleic acids encoding delta-12 and delta-15 fatty acid desaturase proteins that affect fatty acid composition in plants, polypeptides produced from such nucleic acids and plants expressing such nucleic acids.

Background of the Invention

Many breeding studies have been conducted to improve the fatty acid profile of *Brassica* varieties. Pleines and Freidt, Fat Sci. Technol., 90(5), 167-171 (1988) report plant lines with reduced $C_{18:3}$ levels (2.5-5.8%) combined with high oleic content (73-79%). Rakow and McGregor, J. Amer. Oil Chem. Soc., 50, 400-403 (Oct. 1973) discuss problems associated with selecting mutants for linoleic and linolenic acids. In Can. J. Plant Sci., 68, 509-511 (Apr. 1988) Stellar summer rape producing seed oil with 3% linolenic acid and 28% linoleic acid is disclosed. Roy and Tarr, Z. Pflanzenzuchtg, 95(3), 201-209 (1985) report transfer of genes through an interspecific cross from *Brassica juncea* into *Brassica napus* resulting in a reconstituted line combining high linoleic with low linolenic acid content. Roy and Tarr, Plant Breeding, 98, 89-96 (1987) discuss prospects for development of *B. napus L.* having improved linolenic and linolenic acid content. European Patent application 323,753 published July 12, 1989 discloses seeds and oils having greater than 79% oleic acid combined with less than 3.5% linolenic acid. Canvin, Can. J. Botany, 43, 63-69 (1965) discusses the effect of temperature on the fatty acid composition of oils from several seed crops including rapeseed.

Mutations typically are induced with extremely high doses of radiation and/or chemical mutagens (Gaul, H. Radiation Botany (1964) 4:155-232). High

dose levels which exceed LD_{50}, and typically reach LD_{90}, led to maximum achievable mutation rates. In mutation breeding of *Brassica* varieties, high levels of chemical mutagens alone or combined with radiation have induced a limited number of fatty acid mutations (Rakow, G.Z. Pflanzenzuchtg (1973) 69:62-82). The low α-linolenic acid mutation derived from the Rakow mutation breeding program did not have direct commercial application because of low seed yield. The first commercial cultivar using the low α-linolenic acid mutation derived in 1973 was released in 1988 as the variety Stellar (Scarth, R. et al., Can. J. Plant Sci. (1988) 68:509-511). Stellar was 20% lower yielding than commercial cultivars at the time of its release.

Alterations in fatty acid composition of vegetable oils is desirable for meeting specific food and industrial uses. For example, *Brassica* canola varieties with increased monounsaturate levels (oleic acid) in the seed oil, and products derived from such oil, would improve lipid nutrition. Canola lines which are low in polyunsaturated fatty acids and high in oleic acid tend to have higher oxidative stability, which is a useful trait for the retail food industry. Useful traits of vegetable oils for industrial uses like lubrication fluids include desirable low temperature behavior such as low pour point and low cloud point along with very high oxidative stability.

Delta-12 fatty acid desaturase (also known as oleic desaturase) is involved in the enzymatic conversion of oleic acid to linoleic acid. Delta-15 fatty acid desaturase (also known as linoleic acid desaturase) is involved in the enzymatic conversion of linoleic acid to α-linolenic acid. A microsomal delta-12 desaturase has been cloned and characterized using T-DNA tagging. Okuley, et al., Plant Cell 6:147-158 (1994). The nucleotide sequences of higher plant genes encoding microsomal delta-12 fatty acid desaturase are described in Lightner et al., WO94/11516. Sequences of higher plant genes encoding microsomal and plastid delta-15 fatty acid desaturases are disclosed in Yadav, N., et al., Plant Physiol., 103:467-476 (1993), WO 93/11245 and Arondel, V. et al., Science, 258:1353-1355 (1992).

Summary of the Invention

Triacylglycerols containing fatty acids with heterogenous chain lengths and with high monounsaturate levels can provide useful traits for industrial purposes. Plants with fatty acid compositions that have high monounsaturate levels and heterogenous chain lengths would provide a source of industrial oils for uses such as lubrication.

In one aspect, the invention features a *Brassica* plant, and progeny thereof, producing seeds having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition. The oleic acid and eicosenoic acid content of the seeds is at least about 37% and at least about 14%, based on total fatty acid composition, respectively. The saturated fatty acid content of such seeds is less than 7% and the polyunsaturated fatty acid content is less than about 11%.

In some embodiments, the plants have a monounsaturated fatty acid content of from about 85% to about 90% and an erucic acid content of at least about 15% based on total fatty acid composition. In such plants, the oleic acid content can be at least about 42% and in particular, from about 47% to about 56% based on total fatty acid composition. The erucic acid content is from about 17% to about 31%, and the eicosenoic acid content is from about 15% to about 21%.

The invention also features a *Brassica* seed oil having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition. Such oils can have an oleic acid and eicosenoic acid content of at least about 14% and 37%, respectively, based on total fatty acid composition. The saturated fatty acid content can be less than about 7%, e.g., less than about 4% or about 2 to 4%. The polyunsaturated fatty acid content is less than about 11% and in particular embodiments, less than 9%, based on total fatty acid composition. The α-linolenic acid content can be about 1% to about 2%.

In some embodiments, the *Brassica* seed oil contains a long chain monounsaturated fatty acid content of from about 85% to about 90%. In such oils,

the oleic acid content is at least about 42%, and in particular embodiments, is from about 47% to about 56%, based on total fatty acid composition. The erucic acid and eicosenoic acid content is from about 17% to about 31% and from about 15% to about 21%, respectively, based on total fatty acid composition. Also featured is a *Brassica* seed oil having a long chain monounsaturated fatty acid content of at least about 82%, wherein the sum of the nervonic acid, erucic acid and eicosenoic acid content is from about 50% to about 66% based on total fatty acid composition. Such a seed oil can have an oleic acid content from about 25% to about 30%.

The invention also features a method of producing plants having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15%, based on total fatty acid composition. The methods include crossing a first plant line with a second plant line and selecting progeny with the desired fatty acid composition. The first plant line has an erucic acid content of at least about 45%. The second plant line has an oleic acid content of at least about 84%.

The invention also features a method of making a vegetable oil. The method comprises the steps of crushing *Brassica* seeds having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition, and extracting the vegetable oil from the crushed seeds. The method can also include the steps of refining and bleaching the oil, and deodorizing the oil.

The invention also features a lubricant or hydraulic fluid comprising a *Brassica* oil having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition, and an additive. The additive can be an antioxidant, a rust inhibitor, a corrosion inhibitor, a pour point depressant, an anti-foam additive, a colorant and a detergent. The additive can be present in an amount from about 0.01% to about 20% by weight.

Brief Description of the Sequence Listing

SEQ ID NO:1 shows the DNA sequence for the coding region of a wild type *Brassica* Fad2-D gene. SEQ ID NO:2 is the deduced amino acid sequence for SEQ ID NO:1.

SEQ ID NO:3 shows the DNA sequence for the coding region of the IMC 129 mutant *Brassica* Fad2-D gene. SEQ ID NO:4 is the deduced amino acid sequence for SEQ ID NO:3.

SEQ ID NO:5 shows the DNA sequence for the coding region of a wild type *Brassica* Fad2-F gene. SEQ ID NO:6 is the deduced amino acid sequence for SEQ ID NO:5.

SEQ ID NO:7 shows the DNA sequence for the coding region of the Q508 mutant *Brassica* Fad2-F gene. SEQ ID NO:8 is the deduced amino acid sequence for SEQ ID NO:7.

SEQ ID NO:9 shows the DNA sequence for the coding region of the Q4275 mutant *Brassica* Fad2-F gene. SEQ ID NO:10 is the deduced amino acid sequence for SEQ ID NO:9.

Brief Description of the Figures

Figure 1 is a histogram showing the frequency distribution of seed oil oleic acid ($C_{18:1}$) content in a segregating population of a Q508 X Westar cross. The bar labeled WSGA 1A represents the $C_{18:1}$ content of the Westar parent. The bar labeled Q508 represents the $C_{18:1}$ content of the Q508 parent.

Figure 2 shows the nucleotide sequences for a *Brassica* Fad2-D wild type gene (Fad2-D wt), IMC129 mutant gene (Fad2-D GA316 IMC129), Fad2-F wild type gene (Fad2-F wt), Q508 mutant gene (Fad2-F TA515 Q508) and Q4275 mutant gene (Fad2-F GA908 Q4275).

Figure 3 shows the deduced amino acid sequences for the polynucleotides of Figure 2.

Figure 4 is a schematic of a breeding procedure used to produce *Brassica* plants having a high erucic acid and a high oleic acid content.

Detailed Description

All percent fatty acids herein are percent by weight of the oil of which the fatty acid is a component.

As used herein, a "line" is a group of plants that display little or no genetic variation between individuals for at least one trait. Such lines may be created by several generations of self-pollination and selection, or vegetative propagation from a single parent using tissue or cell culture techniques. As used herein, the term "variety" refers to a line which is used for commercial production.

The term "mutagenesis" refers to the use of a mutagenic agent to induce random genetic mutations within a population of individuals. The treated population, or a subsequent generation of that population, is then screened for usable trait(s) that result from the mutations. A "population" is any group of individuals that share a common gene pool. As used herein "M_0" is untreated seed. As used herein, "M_1" is the seed (and resulting plants) exposed to a mutagenic agent, while "M_2" is the progeny (seeds and plants) of self-pollinated M_1 plants, "M_3" is the progeny of self-pollinated M_2 plants, and "M_4" is the progeny of self-pollinated M_3 plants. "M_5" is the progeny of self-pollinated M_4 plants. "M_6", "M_7", etc. are each the progeny of self-pollinated plants of the previous generation. The term "selfed" as used herein means self-pollinated.

"Stability" or "stable" as used herein means that with respect to a given fatty acid component, the component is maintained from generation to generation for at least two generations and preferably at least three generations at substantially the same level, e.g., preferably $\pm$5%. The method of invention is capable of creating lines with improved fatty acid compositions stable up to $\pm$5% from generation to generation. The above stability may be affected by temperature, location, stress and time of planting. Thus, comparison of fatty acid profiles should be made from seeds produced under similar growing conditions. Stability may be measured based on knowledge of prior generation.

Intensive breeding has produced certain *Brassica* plants whose seed oil contains less than 2% erucic acid. The same varieties have also been bred so that

the defatted meal contains less than 30 μmol glucosinolates/gram. "Canola" as used herein refers to plant seeds or oils which contain less than 2% erucic acid ($C_{22:1}$), and result in a defatted meal with less than 30 μmol glucosinolates/gram.

Applicants have discovered plants with mutations in a delta-12 fatty acid desaturase gene. Such plants have useful alterations in the fatty acid compositions of the seed oil. Such mutations confer, for example, an elevated oleic acid content, a decreased, stabilized linoleic acid content, or both elevated oleic acid and decreased, stabilized linoleic acid content.

Applicants have further discovered plants with mutations in a delta-15 fatty acid desaturase gene. Such plants have useful alterations in the fatty acid composition of the seed oil, e.g., a decreased, stabilized level of α-linolenic acid.

Applicants have further discovered isolated nucleic acid fragments (polynucleotides) comprising sequences that carry mutations within the coding sequence of delta-12 or delta-15 fatty acid desaturases. The mutations confer desirable alterations in fatty acid levels in the seed oil of plants carrying such mutations. Delta-12 fatty acid desaturase is also known as omega-6 fatty acid desaturase and is sometimes referred to herein as Fad2 or 12-DES. Delta-15 fatty acid desaturase is also known on omega-3 fatty acid desaturase and is sometimes referred to herein as Fad3 or 15-DES.

A nucleic acid fragment of the invention may be in the form of RNA or in the form of DNA, including cDNA, synthetic DNA or genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded, can be either the coding strand or non-coding strand. An RNA analog may be, for example, mRNA or a combination of ribo- and deoxyribonucleotides. Illustrative examples of a nucleic acid fragment of the invention are the mutant sequences shown in Fig. 3.

A nucleic acid fragment of the invention contains a mutation in a microsomal delta-12 fatty acid desaturase coding sequence or a mutation in a microsomal delta-15 fatty acid desaturase coding sequence. Such a mutation renders the resulting desaturase gene product non-functional in plants, relative to

the function of the gene product encoded by the wild-type sequence. The non-functionality of the delta-12 desaturase gene product can be inferred from the decreased level of reaction product (linoleic acid) and increased level of substrate (oleic acid) in plant tissues expressing the mutant sequence, compared to the corresponding levels in plant tissues expressing the wild-type sequence. The non-functionality of the delta-15 desaturase gene product can be inferred from the decreased level of reaction product (α-linolenic acid) and the increased level of substrate (linoleic acid) in plant tissues expressing the mutant sequence, compared to the corresponding levels in plant tissues expressing the wild-type sequence.

A nucleic acid fragment of the invention may comprise a portion of the coding sequence, e.g., at least about 10 nucleotides, provided that the fragment contains at least one mutation in the coding sequence. The length of a desired fragment depends upon the purpose for which the fragment will be used, e.g., PCR primer, site-directed mutagenesis and the like. In one embodiment, a nucleic acid fragment of the invention comprises the full length coding sequence of a mutant delta-12 or mutant delta-15 fatty acid desaturase, e.g., the mutant sequences of Fig. 3. In other embodiments, a nucleic acid fragment is about 20 to about 50 nucleotides (or base pairs, bp), or about 50 to about 500 nucleotides, or about 500 to about 1200 nucleotides in length.

Desirable alterations in fatty acid levels in the seed oil of plants can be produced using a ribozyme. Ribozyme molecules designed to cleave delta-12 or delta-15 desaturase mRNA transcripts can be used to prevent expression of delta-12 or delta-15 desaturases. While various ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy desaturase mRNAs, hammerhead ribozymes are particularly useful. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target RNA contain a 5'-UG-3' nucleotide sequence. The construction and production of hammerhead ribozymes is well known in the art. See, for example, U.S. Patent No. 5,254,678. Hammerhead ribozyme sequences can be embedded in a stable RNA such as a transfer RNA

(tRNA) to increase cleavage efficiency *in vivo*. Perriman, R. et al., Proc. Natl. Acad. Sci. USA, 92(13):6175-6179 (1995); de Feyter, R. and Gaudron, J., Methods in Molecular Biology, Vol. 74, Chapter 43, "Expressing Ribozymes in Plants", Edited by Turner, P.C, Humana Press Inc., Totowa, NJ. RNA endoribonucleases such as the one that occurs naturally in *Tetrahymena thermophila*, and which have been described extensively by Cech and collaborators are also useful. See, for example, U.S. Patent No. 4,987,071.

A mutation in a nucleic acid fragment of the invention may be in any portion of the coding sequence that renders the resulting gene product non-functional. Suitable types of mutations include, without limitation, insertions of nucleotides, deletions of nucleotides, or transitions and transversions in the wild-type coding sequence. Such mutations result in insertions of one or more amino acids, deletions of one or more amino acids, and non-conservative amino acid substitutions in the corresponding gene product. In some embodiments, the sequence of a nucleic acid fragment may comprise more than one mutation or more than one type of mutation.

Insertion or deletion of amino acids in a coding sequence may, for example, disrupt the conformation of essential alpha-helical or beta-pleated sheet regions of the resulting gene product. Amino acid insertions or deletions may also disrupt binding or catalytic sites important for gene product activity. It is known in the art that the insertion or deletion of a larger number of contiguous amino acids is more likely to render the gene product non-functional, compared to a smaller number of inserted or deleted amino acids. Non-conservative amino acid substitutions may replace an amino acid of one class with an amino acid of a different class. Non-conservative substitutions may make a substantial change in the charge or hydrophobicity of the gene product. Non-conservative amino acid substitutions may also make a substantial change in the bulk of the residue side chain, e.g., substituting an alanyl residue for a isoleucyl residue.

Examples of non-conservative substitutions include the substitution of a basic amino acid for a non-polar amino acid, or a polar amino acid for an acidic

amino acid. Because there are only 20 amino acids encoded in a gene, substitutions that result in a non-functional gene product may be determined by routine experimentation, incorporating amino acids of a different class in the region of the gene product targeted for mutation.

Preferred mutations are in a region of the nucleic acid encoding an amino acid sequence motif that is conserved among delta-12 fatty acid desaturases or delta-15 fatty acid desaturases, such as a His-Xaa-Xaa-Xaa-His motif (Tables 1-3). An example of a suitable region has a conserved HECGH motif that is found, for example, in nucleotides corresponding to amino acids 105 to 109 of the *Arabidopsis* and *Brassica* delta-12 desaturase sequences, in nucleotides corresponding to amino acids 101 to 105 of the soybean delta-12 desaturase sequence and in nucleotides corresponding to amino acids 111 to 115 of the maize delta-12 desaturase sequence. See e.g., WO 94/115116; Okuley et al., Plant Cell 6:147-158 (1994). The one letter amino acid designations used herein are described in Alberts, B. et al., Molecular Biology of the Cell, 3rd edition, Garland Publishing, New York, 1994. Amino acids flanking this motif are also highly conserved among delta-12 and delta-15 desaturases and are also suitable candidates for mutations in fragments of the invention.

An illustrative embodiment of a mutation in a nucleic acid fragment of the invention is a Glu to Lys substitution in the HECGH motif of a *Brassica* microsomal delta-12 desaturase sequence, either the D form or the F form. This mutation results in the sequence HECGH being changed to HKCGH as seen by comparing SEQ ID NO:2 (wild-type D form) to SEQ ID NO:4 (mutant D form). A similar mutation in other Fad-2 sequences is contemplated to result in a non-functional gene product.

A similar motif may be found at amino acids 101 to 105 of the *Arabidopsis* microsomal delta-15 fatty acid desaturase, as well as in the corresponding rape and soybean desaturases (Table 5). See, e.g., WO 93/11245; Arondel, V. et al., Science, 258:1153-1155 (1992); Yadav, N. et al., Plant Physiol., 103:467-476 (1993). Plastid delta-15 fatty acids have a similar motif (Table 5).

Among the types of mutations in an HECGH motif that render the resulting gene product non-functional are non-conservative substitutions. An illustrative example of a non-conservative substitution is substitution of a glycine residue for either the first or second histidine. Such a substitution replaces a charged residue (histidine) with a non-polar residue (glycine). Another type of mutation that renders the resulting gene product non-functional is an insertion mutation, e.g., insertion of a glycine between the cysteine and glutamic acid residues in the HECGH motif.

Other regions having suitable conserved amino acid motifs include the HRRHH motif shown in Table 2, the HRTHH motif shown in Table 6 and the HVAHH motif shown in Table 3. See, e.g., WO 94/115116; Hitz, W. et al., Plant Physiol., 105:635-641 (1994); Okuley, J., et al., supra; and Yadav, N. et al., supra. An illustrative example of a mutation in the region shown in Table 3 is a mutation at nucleotides corresponding to the codon for glycine (amino acid 303 of *B. napus*). A non-conservative Gly to Glu substitution results in the amino acid sequence DRDYGILNKV being changed to sequence DRDYEILNKV (compare wild-type F form SEQ ID NO:6 to mutant Q4275 SEQ ID NO:10, Fig. 3).

Another region suitable for a mutation in a delta-12 desaturase sequence contains the motif KYLNNP at nucleotides corresponding to amino acids 171 to 175 of the *Brassica* desaturase sequence. An illustrative example of a mutation is this region is a Leu to His substitution, resulting in the amino acid sequence (Table 4) KYHNN (compare wild-type Fad2-F SEQ ID NO:6 to mutant SEQ ID NO:8). A similar mutation in other Fad-2 amino acid sequences is contemplated to result in a non-functional gene product.

TABLE 1

Alignment of Amino Acid Sequences from Microsomal Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
Arabidopsis thaliana	100-129	IWVIAHECGH HAFSDYQWLD DTVGLIFHSF
Glycine max	96-125	VWVIAHECGH HAFSKYQWVD DVVGLTLHST
Zea mays	106-135	VWVIAHECGH HAFSDYSLLD DVVGLVLHSS
Ricinus communis[a]	1- 29	WVMAHDCGH HAFSDYQLLD DVVGLILHSC
Brassica napus D	100-128	VWVIAHECGH HAFSDYQWLD DTVGLIFHS
Brassica napus F	100-128	VWVIAHECGH HAFSDYQWLD DTVGLIFHS

[a] from plasmid pRF2-1C

TABLE 2

Alignment of Amino Acid Sequences from Microsomal Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
Arabidopsis thaliana	130-158	LLVPYFSWKY SHRRHHSNTG SLERDEVFV
Glycine max	126-154	LLVPYFSWKI SHRRHHSNTG SLDRDEVFV
Zea mays	136-164	LMVPYFSWKY SHRRHHSNTG SLERDEVFV
Ricinus communis[a]	30- 58	LLVPYFSWKH SHRRHHSNTG SLERDEVFV
Brassica napus D	130-158	LLVPYFSWKY SHRRHHSNTG SLERDEVFV
Brassica napus F	130-158	LLVPYFSWKY SHRRHHSNTG SLERDEVFV

[a] from plasmid pRF2-1C

TABLE 3

Alignment of Amino Acid Sequences from Microsomal Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
Arabidopsis thaliana	298-333	DRDYGILNKV FHNITDTHVA HHLFSTMPHY NAMEAT
Glycine max	294-329	DRDYGILNKV FHHITDTHVA HHLFSTMPHY HAMEAT
Zea mays	305-340	DRDYGILNRV FHNITDTHVA HHLFSTMPHY HAMEAT
Ricinus communis[a]	198-224	DRDYGILNKV FHNITDTQVA HHLF TMP
Brassica napus D	299-334	DRDYGILNKV FHNITDTHVA HHLFSTMPHY HAMEAT
Brassica napus F	299-334	DRDYGILNKV FHNITDTHVA HHLFSTMPHY HAMEAT

[a] from plasmid pRF2-1C

TABLE 4

Alignment of Conserved Amino Acids from Microsomal Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
Arabidopsis thaliana	165-180	IKWYGKYLNN PLGRIM
Glycine max	161-176	VAWFSLYLNN PLGRAV
Zea mays	172-187	PWYTPYVYNN PVGRVV
Ricinus communis[a]	65- 80	IRWYSKYLNN PPGRIM
Brassica napus D	165-180	IKWYGKYLNN PLGRTV
Brassica napus F	165-180	IKWYGKYLNN PLGRTV

[a] from plasmid pRF2-1C

TABLE 5

Alignment of Conserved Amino Acids from Plastid and Microsomal Delta-15 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
Arabidopsis thaliana[a]	156-177	WALFVLGHD CGHGSFSNDP KLN
Brassica napus[a]	114-135	WALFVLGHD CGHGSFSNDP RLN
Glycine max[a]	164-185	WALFVLGHD CGHGSFSNNS KLN
Arabidopsis thaliana	94-115	WAIFVLGHD CGHGSFSDIP LLN
Brassica napus	87-109	WALFVLGHD CGHGSFSNDP RLN
Glycine max	93-114	WALFVLGHD CGHGSFSDSP PLN

[a] Plastid sequences

TABLE 6

Alignment of Conserved Amino Acids from Plastid and Microsomal Delta-15 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
A. thaliana[a]	188-216	ILVPYHGWRI SHRTHHQNHG HVENDESWH
B. napus[a]	146-174	ILVPYHGWRI SHRTHHQNHG HVENDESWH
Glycine max[a]	196-224	ILVPYHGWRI SHRTHHQHHG HAENDESWH
A. thaliana	126-154	ILVPYHGWRI SHRTHHQNHG HVENDESWV
Brassica napus	117-145	ILVPYHGWRI SHRTHHQNHG HVENDESWV
Glycine max	125-153	ILVPYHGWRI SHRTHHQNHG HIEKDESWV

[a] Plastid sequences

The conservation of amino acid motifs and their relative positions indicates that regions of a delta-12 or delta-15 fatty acid desaturase that can be mutated in one species to generate a non-functional desaturase can be mutated in the corresponding region from other species to generate a non-functional delta-12 desaturase or delta-15 desaturase gene product in that species.

Mutations in any of the regions of Tables 1-6 are specifically included within the scope of the invention and are substantially identical to those mutations exemplified herein, provided that such mutation (or mutations) renders the resulting desaturase gene product non-functional, as discussed hereinabove.

A nucleic acid fragment containing a mutant sequence can be generated by techniques known to the skilled artisan. Such techniques include, without limitation, site-directed mutagenesis of wild-type sequences and direct synthesis using automated DNA synthesizers.

A nucleic acid fragment containing a mutant sequence can also be generated by mutagenesis of plant seeds or regenerable plant tissue by, e.g., ethyl methane sulfonate, X-rays or other mutagens. With mutagenesis, mutant plants having the desired fatty acid phenotype in seeds are identified by known techniques and a nucleic acid fragment containing the desired mutation is isolated from genomic DNA or RNA of the mutant line. The site of the specific mutation is then

determined by sequencing the coding region of the delta-12 desaturase or delta-15 desaturase gene. Alternatively, labeled nucleic acid probes that are specific for desired mutational events can be used to rapidly screen a mutagenized population.

The disclosed method may be applied to all oilseed *Brassica* species, and to both Spring and Winter maturing types within each species. Physical mutagens, including but not limited to X-rays, UV rays, and other physical treatments which cause chromosome damage, and other chemical mutagens, including but not limited to ethidium bromide, nitrosoguanidine, diepoxybutane etc. may also be used to induce mutations. The mutagenesis treatment may also be applied to other stages of plant development, including but not limited to cell cultures, embryos, microspores and shoot apices.

"Stable mutations" as used herein are defined as M_5 or more advanced lines which maintain a selected altered fatty acid profile for a minimum of three generations, including a minimum of two generations under field conditions, and exceeding established statistical thresholds for a minimum of two generations, as determined by gas chromatographic analysis of a minimum of 10 randomly selected seeds bulked together. Alternatively, stability may be measured in the same way by comparing to subsequent generations. In subsequent generations, stability is defined as having similar fatty acid profiles in the seed as that of the prior or subsequent generation when grown under substantially similar conditions.

Mutation breeding has traditionally produced plants carrying, in addition to the trait of interest, multiple, deleterious traits, e.g., reduced plant vigor and reduced fertility. Such traits may indirectly affect fatty acid composition, producing an unstable mutation; and/or reduce yield, thereby reducing the commercial utility of the invention. To eliminate the occurrence of deleterious mutations and reduce the load of mutations carried by the plant, a low mutagen dose is used in the seed treatments to create an LD30 population. This allows for the rapid selection of single gene mutations for fatty acid traits in agronomic backgrounds which produce acceptable yields.

The seeds of several different fatty acid lines have been deposited with the American Type Culture Collection and have the following accession numbers.

Line	Accession No.	Deposit Date
A129.5	40811	May 25, 1990
A133.1	40812	May 25, 1990
M3032.1	75021	June 7, 1991
M3062.8	75025	June 7, 1991
M3028.10	75026	June 7, 1991
IMC130	75446	April 16, 1993
Q4275	97569	May 10, 1996

In some plant species or varieties more than one form of endogenous microsomal delta-12 desaturase may be found. In amphidiploids, each form may be derived from one of the parent genomes making up the species under consideration. Plants with mutations in both forms have a fatty acid profile that differs from plants with a mutation in only one form. An example of such a plant is *Brassica napus* line *Q508*, a doubly-mutagenized line containing a mutant D-form of delta-12 desaturase (SEQ ID NO:3) and a mutant F-form of delta-12 desaturase (SEQ ID NO:7). Another example is line Q4275, which contains a mutant D-form of delta-12 desaturase (SEQ ID NO:3) and a mutant F-form of delta-12 desaturase (SEQ ID NO:9). See Figs. 2-3.

Preferred host or recipient organisms for introduction of a nucleic acid fragment of the invention are the oil-producing species, such as soybean (*Glycine max*), rapeseed (e.g., *Brassica napus*, *B. rapa* and *B. juncea*), sunflower (*Helianthus annus*), castor bean (*Ricinus communis*), corn (*Zea mays*), and safflower (*Carthamus tinctorius*).

A nucleic acid fragment of the invention may further comprise additional nucleic acids. For example, a nucleic acid encoding a secretory or leader amino acid sequence can be linked to a mutant desaturase nucleic acid fragment such that the secretory or leader sequence is fused in-frame to the amino terminal end of a mutant delta-12 or delta-15 desaturase polypeptide. Other nucleic acid

fragments are known in the art that encode amino acid sequences useful for fusing in-frame to the mutant desaturase polypeptides disclosed herein. See, e.g., U.S. 5,629,193 incorporated herein by reference. A nucleic acid fragment may also have one or more regulatory elements operably linked thereto.

The present invention also comprises nucleic acid fragments that selectively hybridize to mutant desaturase sequences. Such a nucleic acid fragment typically is at least 15 nucleotides in length. Hybridization typically involves Southern analysis (Southern blotting), a method by which the presence of DNA sequences in a target nucleic acid mixture are identified by hybridization to a labeled oligonucleotide or DNA fragment probe. Southern analysis typically involves electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane support for analysis with a radiolabeled, biotinylated, or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook et al., (1989) *Molecular Cloning*, second edition, Cold Spring Harbor Laboratory, Plainview; NY.

A nucleic acid fragment can hybridize under moderate stringency conditions or, preferably, under high stringency conditions to a mutant desaturase sequence. High stringency conditions are used to identify nucleic acids that have a high degree of homology to the probe. High stringency conditions can include the use of low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC); 0.1% sodium lauryl sulfate (SDS) at 50-65°C. Alternatively, a denaturing agent such as formamide can be employed during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is the use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Moderate stringency conditions refers to hybridization conditions used to identify nucleic acids that have a lower degree of identity to the probe than do nucleic acids identified under high stringency conditions. Moderate stringency conditions can include the use of higher ionic strength and/or lower temperatures for washing of the hybridization membrane, compared to the ionic strength and temperatures used for high stringency hybridization. For example, a wash solution comprising 0.060 M NaCl/0.0060 M sodium citrate (4X SSC) and 0.1% sodium lauryl sulfate (SDS) can be used at 50°C, with a last wash in 1X SSC, at 65°C. Alternatively, a hybridization wash in 1X SSC at 37°C can be used.

Hybridization can also be done by Northern analysis (Northern blotting), a method used to identify RNAs that hybridize to a known probe such as an oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ^{32}P, by biotinylation or with an enzyme. The RNA to be analyzed can be usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., *supra*.

A polypeptide of the invention comprises an isolated polypeptide having a mutant amino acid sequence, as well as derivatives and analogs thereof. See, e.g., the mutant amino acid sequences of Fig. 3. By "isolated" is meant a polypeptide that is expressed and produced in an environment other than the environment in which the polypeptide is naturally expressed and produced. For example, a plant polypeptide is isolated when expressed and produced in bacteria or fungi. A polypeptide of the invention also comprises variants of the mutant desaturase polypeptides disclosed herein, as discussed above.

In one embodiment of the claimed invention, a plant contains both a delta-12 desaturase mutation and a delta-15 desaturase mutation. Such plants can have a fatty acid composition comprising very high oleic acid and very low alpha-linolenic acid levels. Mutations in delta-12 desaturase and delta-15 desaturase may

be combined in a plant by making a genetic cross between delta-12 desaturase and delta-15 desaturase single mutant lines. A plant having a mutation in delta-12 fatty acid desaturase is crossed or mated with a second plant having a mutation in delta-15 fatty acid desaturase. Seeds produced from the cross are planted and the resulting plants are selfed in order to obtain progeny seeds. These progeny seeds are then screened in order to identify those seeds carrying both mutant genes.

Alternatively, a line possessing either a delta-12 desaturase or a delta-15 desaturase mutation can be subjected to mutagenesis to generate a plant or plant line having mutations in both delta-12 desaturase and delta-15 desaturase. For example, the IMC 129 line has a mutation in the coding region (Glu_{106} to Lys_{106}) of the D form of the microsomal delta-12 desaturase structural gene. Cells (e.g., seeds) of this line can be mutagenized to induce a mutation in a delta-15 desaturase gene, resulting in a plant or plant line carrying a mutation in a delta-12 fatty acid desaturase gene and a mutation in a delta-15 fatty acid desaturase gene.

Progeny includes descendants of a particular plant or plant line, e.g., seeds developed on an instant plant are descendants. Progeny of an instant plant include seeds formed on F_1, F_2, F_3, and subsequent generation plants, or seeds formed on BC_1, BC_2, BC_3 and subsequent generation plants.

Plants according to the invention preferably contain an altered fatty acid composition. For example, oil obtained from seeds of such plants may have from about 69 to about 90% oleic acid, based on the total fatty acid composition of the seed. Such oil preferably has from about 74 to about 90% oleic acid, more preferably from about 80 to about 90% oleic acid. In some embodiments, oil obtained from seeds produced by plants of the invention may have from about 2.0% to about 5.0% saturated fatty acids, based on total fatty acid composition of the seeds. In some embodiments, oil obtained from seeds of the invention may have from about 1.0% to about 14.0% linoleic acid, or from about 0.5% to about 10.0% α-linolenic acid.

Oil composition typically is analyzed by crushing and extracting fatty acids from bulk seed samples (e.g., 10 seeds). Fatty acid triglycerides in the seed

are hydrolyzed and converted to fatty acid methyl esters. Those seeds having an altered fatty acid composition may be identified by techniques known to the skilled artisan, e.g., gas-liquid chromatography (GLC) analysis of a bulked seed sample, single seed or a single half-seed. Half-seed analysis is well known in the art to be useful because the viability of the embryo is maintained and thus those seeds having a desired fatty acid profile may be planted to form the next generation. However, half-seed analysis is also known to be an inaccurate representation of genotype of the seed being analyzed. Bulk seed analysis typically yields a more accurate representation of the fatty acid profile of a given genotype. Half-seed analysis of a population of seeds is, however, a reliable indicator of the likelihood of obtaining a desired fatty acid profile. Fatty acid composition can also be determined on larger samples, e.g., oil obtained by pilot plant or commercial scale refining, bleaching and deodorizing of endogenous oil in the seeds.

The nucleic acid fragments of the invention can be used as markers in plant genetic mapping and plant breeding programs. Such markers may include restriction fragment length polymorphism (RFLP), random amplification polymorphism detection (RAPD), polymerase chain reaction (PCR) or self-sustained sequence replication (3SR) markers, for example. Marker-assisted breeding techniques may be used to identify and follow a desired fatty acid composition during the breeding process. Marker-assisted breeding techniques may be used in addition to, or as an alternative to, other sorts of identification techniques. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence containing a desired mutation in delta-12 desaturase or delta-15 desaturase.

Methods according to the invention are useful in that the resulting plants and plant lines have desirable seed fatty acid compositions as well as superior agronomic properties compared to known lines having altered seed fatty acid composition. Superior agronomic characteristics include, for example, increased seed germination percentage, increased seedling vigor, increased resistance to

seedling fungal diseases (damping off, root rot and the like), increased yield, and improved standability.

In another aspect, *Brassica* plants producing seeds having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15%, based on total fatty acid composition, are featured. As used herein, "long chain" refers to carbon chains of 16 and greater, e.g., chains of 16 to 24 carbons. The long chain monounsaturated fatty acid content is distributed primarily among oleic acid, eicosenoic acid and erucic acid. The heterogenous nature of the long chain monounsaturated fatty acids in the seed oil triacylglycerols confers desirable properties to the oil. The levels of total saturated fatty acids and/or the total polyunsaturated fatty acids can be decreased in order to increase the long chain monounsaturate content, i.e., oleic acid, eicosenoic acid, erucic acid and nervonic acid.

High oleic acid lines described herein can be crossed to high erucic acid lines to produce *Brassica* plants having a high long chain monounsaturated fatty acid content within their seeds. Suitable high oleic acid lines are described, for example, in Example 5 and Table 17, and have an oleic acid content of about 82% to about 85%, based on total fatty acid composition. Suitable high erucic acid lines have an erucic acid content of about 45% or greater, based on total fatty acid composition. *Brassica* plant line HEC01 is a high erucic acid line that is particularly useful and is sold under the trade name Hero. Other high erucic acid varieties are also known, such as varieties designated Venus, Mercury, Neptune, S89-3673, Dwarf Essex, Reston, Bridger or R-500. McVetty, P.B.E. et al., Can. J. Plant Sci., 76(2):341-342 (1996); Scarth, R. et al., Can. J. Plant Sci., 75(1):205-206 (1995); and McVetty, P.B.E. et al., Can. J. Plant Sci., 76(2):343-344 (1996).

Seeds of the invention can have an oleic acid and eicosenoic acid content of at least about 37% and 14%, respectively, based on total fatty acid composition. The total saturated fatty acid content is less than about 7%. As used herein, "total saturated fatty acid content" refers to the total of myristate (14:0), palmitate (16:0), stearate (18:0), arachidate (20:0), behenate (22:0) and lignocerate

(24:0). The total polyunsaturated content is less than about 11% based on total fatty acid composition. As used herein, "total polyunsaturated fatty acid content" refers to the sum of linoleic (18:2), α-linolenic (18:3), and eicosadienoic (20:2) fatty acids as a percentage of the total fatty acid content.

In some embodiments, the long chain monounsaturate content in seeds is from about 85% to about 90%. The oleic acid content within these seeds is about 42% or greater, and preferably from about 47% to about 56%. The erucic acid and eicosenoic acid content are from about 17% to about 31% and from about 15% to about 21%, respectively.

In some embodiments, the long chain monounsaturate content in seeds is from about 82% to about 90%. The oleic acid content within these seeds is about 25% to about 33%. The erucic acid and eicosenoic acid content are from about 44% to about 50% and from about 10% to about 13%, respectively.

Seed oils having a long chain monounsaturated content of at least about 82% and an erucic acid content of at least about 15%, based on total fatty acid composition, are also featured. These oils can be extracted, for example, from a single line of *Brassica* seeds having a suitable fatty acid composition as described herein. The oleic acid and eicosenoic acid content of these oils is at least about 37% and 14%, respectively, based on total fatty acid composition. The total saturated and polyunsaturated content of these oils is less than about 7% and 11%, respectively. Preferably, the polyunsaturated content is less than about 9%. In some embodiments, the oils have a monounsaturated content of from about 85% to about 90%. The oleic acid content of these oils is at least about 42% and more preferably, from about 47% to about 56%. The oils have an erucic acid content of from about 17% to about 31% and an eicosenoic acid content of from about 15% to about 21%.

In some embodiments, the long chain monounsaturate content in such oils is from about 82% to about 90%, comprising an oleic acid content of about 25% to about 33%, an erucic acid of about 44% to about 50% and an eicosenoic acid content of about 10% to about 13%. The sum of the nervonic acid, erucic

acid and eicosenoic acid contents is such oils can be from about 50% to about 66%.

Alternatively, it is contemplated that oils of the invention can be obtained by mixing high-erucic acid rapeseed oil (HEAR) and an oil having at least about 87% oleic acid, preferably from about 90% to about 95% oleic acid, based on total fatty acid composition. HEAR oil has an erucic acid content of about 49% and an oleic acid content of about 16%.

Oils having a long chain monounsaturated content of at least about 82% unexpectedly have low temperature properties that are desirable for industrial applications such as lubrication. The basis for these properties is not known, but is it possible that the heterogeneous chain lengths of the triacylglycerols in oils of the invention impede orderly packing as the end methyl groups have a mismatch in molecular volume, reducing Van der Waals interactions. The double bond in each fatty acid moiety is present at different carbon positions along the acyl chain, which may disrupt packing and also reduce π-π electronic interactions between adjacent fatty acid chains. The high monounsaturate content is thought to provide improved oxidative stability along with high fluidity characteristics. The low levels of polyunsaturates in oils of the invention also promotes high oxidative stability, since the rates of oxidation of linoleic acid and linolenic acid at 20°C are 12-20 times and 25 times, respectively, larger than the rate of oxidation of oleic acid.

Oxidative stability can be measured with an Oxidative Stability Index Instrument, Omnion, Inc., Rockland, MA, according to AOCS Official Method Cd 12b-92 (revised 1993). The method is an automated replacement for the Active Oxygen Method (AOM) procedure, AOCS Official Method Cd 12-57. Oxidative stability of oils having a long chain monounsaturated content of at least about 82% is from about 40 AOM hours to about 100 AOM hours in the absence of added antioxidants. In comparison, mid-oleic canola oil (about 76% oleic acid) and high erucic acid rapeseed oil have oxidative stabilities of about 38 and 16 AOM hours, respectively, in the absence of added antioxidants.

The oils of the invention have desirable functional properties, e.g., low temperature behavior and a high viscosity index, along with high oxidative stability. The presence of higher molecular weight fatty acids increases the molecular weight of the triacylglycerols, providing the oil with a higher flash point and a higher fire point. The increased molecular weight also improves the viscosity index of the oils. Viscosity index is an arbitrary number that indicates the viscosity change with temperature of a lubricant. The Dean and Davis viscosity index can be calculated from observed viscosities of a lubricant at 40°C and 100°C and can produce values ranging from 0 to values greater than 200. A higher viscosity index value indicates that the viscosity of the oil changes less with a change in temperature. In other words, the higher the viscosity index, the smaller the difference in viscosity between high and low temperatures.

An oil of the invention can be formulated for industrial applications such as engine lubricants or as hydraulic fluids by addition of one or more additives to an oil having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15%, based on total fatty acid composition. For example, a transmission fluid for diesel engines can be made that includes antioxidants, anti-foam additives, anti-wear additives, corrosion inhibitors, dispersants, detergents, and acid neutralizers, or combinations thereof. Hydraulic oil compositions can include antioxidants, anti-rust additives, anti-wear additives, pour point depressants, viscosity-index improvers and anti-foam additives or combinations thereof. Specific formulations will vary depending on the end use of the oil; suitability of a formulation for a specific end use can be assessed using standard techniques.

Typical antioxidants include zinc dithiophosphates, methyl dithiocarbamates, hindered phenols, phenol sulfides, metal phenol sulfides, metal salicylates, aromatic amines, phospho-sulfurized fats and olefins, sulfurized olefins, sulfurized fats and fat derivatives, sulfurized paraffins, sulfurized carboxylic acids, disalieylal-1,2,-propane diamine, 2,4-bis (alkyldithio-1,3,4-thiadiazoles) and dilauryl selenide. Antioxidants are typically present in amounts from about 0.01% to about

5%, based on the weight of the composition. In particular, about 0.01% to about 1.0% of antioxidant is added to an oil of the invention. See U.S. Patent No. 5,451,334 for additional antioxidants.

Rust inhibitors protect surfaces against rust and include, for example, alkylsuccinic type organic acids, and derivatives thereof, alkylthioacetic acids and derivatives thereof, organic amines, organic phosphates, polyhyndric alcohols and sodium and calcium sulphonates. Anti-wear additives adsorb on metal and provide a film that reduces metal-to-metal contact. In general, anti-wear additives include zinc dialkyldithiophosphates, tricresyl phosphate, didodecyl phosphite, sulfurized sperm oil, sulfurized terpenes and zinc dialkyldithiocarbamate, and are used in amounts from about 0.05% to about 4.5%.

Corrosion inhibitors include dithiophosphates and in particular, zinc dithiophosphates, metal sulfonates, metal phenate sulfides, fatty acids, acid phosphate esters and alkyl succinic acids.

Pour point depressants permit flow of the oil composition below the pour point of the unmodified lubricant. Common pour point depressants include polymethacrylates, wax alkylated naphthalene polymers, wax alkylated phenol polymers and chlorinated polymers and are typically present in amounts of about 1% or less. See, for example, U.S. Patent Nos. 5,451,334 and 5,413,725. The viscosity-index can be increased by adding polyisobutylenes, polymethacrylates, polyacrylates, ethylene propylene copolymers, styrene isoprene copolymers, styrene butadiene copolymers and styrene maleic ester copolymers.

Anti-foam additives reduce or prevent the formation of a stable surface foam and are typically present in amounts from about 0.00003% to about 0.05%. Polymethylsiloxanes, polymethacrylates, salts of alkyl alkylene dithiophosphates, amyl acrylate telomer and poly(2-ethylhexylacrylate-co-ethyl acrylate are non-limiting examples of anti-foam additives.

Detergents and dispersants are polar materials that provide a cleaning function. Detergents include metal sulfonates, metal salicylates and metal thiophosponates. Dispersants include polyamine succinimides, hydroxy benzyl

polyamines, polyamine succinamides, polyhydroxy succinic esters and polyamine amide imidazolines.

While the invention is susceptible to various modifications and alternative forms, certain specific embodiments thereof are described in the general methods and examples set forth below. For example the invention may be applied to all *Brassica* species, including *B. rapa*, *B. juncea*, and *B. hirta*, to produce substantially similar results. It should be understood, however, that these examples are not intended to limit the invention to the particular forms disclosed but, instead the invention is to cover all modifications, equivalents and alternatives falling within the scope of the invention. This includes the use of somaclonal variation; physical or chemical mutagenesis of plant parts; anther, microspore or ovary culture followed by chromosome doubling; or self- or cross-pollination to transmit the fatty acid trait, alone or in combination with other traits, to develop new *Brassica* lines.

EXAMPLE 1

Mutagenesis

Seeds of Westar, a Canadian (*Brassica napus*) spring canola variety, were subjected to chemical mutagenesis. Westar is a registered Canadian spring variety with canola quality. The fatty acid composition of field-grown Westar, 3.9% $C_{16:0}$, 1.9% $C_{18:0}$, 67.5% $C_{18:1}$, 17.6% $C_{18:2}$, 7.4% $C_{18:3}$, <2% C20:1 + $C_{22:1}$, has remained stable under commercial production, with $\leq \pm$ 10% deviation, since 1982.

Prior to mutagenesis, 30,000 seeds of *B. napus* cv. Westar seeds were preimbibed in 300-seed lots for two hours on wet filter paper to soften the seed coat. The preimbibed seeds were placed in 80 mM ethylmethanesulfonate (EMS) for four hours. Following mutagenesis, the seeds were rinsed three times in distilled water. The seeds were sown in 48-well flats containing Pro-Mix. Sixty-eight percent of the mutagenized seed germinated. The plants were maintained at 25°C/15°C, 14/10 hr day/night conditions in the greenhouse. At flowering, each plant was individually self-pollinated.

M_2 seed from individual plants were individually catalogued and stored, approximately 15,000 M_2 lines was planted in a summer nursery in Carman, Manitoba. The seed from each selfed plant were planted in 3-meter rows with 6-inch row spacing. Westar was planted as the check variety. Selected lines in the field were selfed by bagging the main raceme of each plant. At maturity, the selfed plants were individually harvested and seeds were catalogued and stored to ensure that the source of the seed was known.

Self-pollinated M_3 seed and Westar controls were analyzed in 10-seed bulk samples for fatty acid composition via gas chromatography. Statistical thresholds for each fatty acid component were established using a Z-distribution with a stringency level of 1 in 10,000. Mean and standard deviation values were determined from the non-mutagenized Westar control population in the field. The upper and lower statistical thresholds for each fatty acid were determined from the mean value of the population $\pm$ the standard deviation, multiplied by the Z-distribution. Based on a population size of 10,000, the confidence interval is 99.99%.

The selected M_3 seeds were planted in the greenhouse along with Westar controls. The seed was sown in 4-inch pots containing Pro-Mix soil and the plants were maintained at 25°C/15°C, 14/10 hr day/night cycle in the greenhouse. At flowering, the terminal raceme was self-pollinated by bagging. At maturity, selfed M_4 seed was individually harvested from each plant, labelled, and stored to ensure that the source of the seed was known.

The M_4 seed was analyzed in 10-seed bulk samples. Statistical thresholds for each fatty acid component were established from 259 control samples using a Z-distribution of 1 in 800. Selected M_4 lines were planted in a field trial in Carman, Manitoba in 3-meter rows with 6-inch spacing. Ten M_4 plants in each row were bagged for self-pollination. At maturity, the selfed plants were individually harvested and the open pollinated plants in the row were bulk harvested. The M_5 seed from single plant selections was analyzed in 10-seed bulk samples and the bulk row harvest in 50-seed bulk samples.

Selected M_5 lines were planted in the greenhouse along with Westar controls. The seed was grown as previously described. At flowering the terminal raceme was self-pollinated by bagging. At maturity, selfed M_6 seed was individually harvested from each plant and analyzed in 10-seed bulk samples for fatty acid composition.

Selected M_6 lines were entered into field trials in Eastern Idaho. The four trial locations were selected for the wide variability in growing conditions. The locations included Burley, Tetonia, Lamont and Shelley (Table 7). The lines were planted in four 3-meter rows with an 8-inch spacing, each plot was replicated four times. The planting design was determined using a Randomized Complete Block Design. The commercial cultivar Westar was used as a check cultivar. At maturity the plots were harvested to determine yield. Yield of the entries in the trial was determined by taking the statistical average of the four replications. The Least Significant Difference Test was used to rank the entries in the randomized complete block design.

TABLE 7

Trial Locations for Selected Fatty Acid Mutants

LOCATION	SITE CHARACTERIZATIONS
BURLEY	Irrigated. Long season. High temperatures during flowering.
TETONIA	Dryland. Short season. Cool temperatures.
LAMONT	Dryland. Short season. Cool temperatures.
SHELLEY	Irrigated. Medium season. High temperatures during flowering.

To determine the fatty acid profile of entries, plants in each plot were bagged for self-pollination. The M_7 seed from single plants was analyzed for fatty acids in ten-seed bulk samples.

To determine the genetic relationships of the selected fatty acid mutants crosses were made. Flowers of M_6 or later generation mutations were used in crossing. F_1 seed was harvested and analyzed for fatty acid composition to determine the mode of gene action. The F_1 progeny were planted in the greenhouse. The resulting plants were self-pollinated, the F_2 seed harvested and analyzed for fatty acid composition for allelism studies. The F_2 seed and parent line seed was planted in the greenhouse, individual plants were self-pollinated. The F_3 seed of individual plants was tested for fatty acid composition using 10-seed bulk samples as described previously.

In the analysis of some genetic relationships dihaploid populations were made from the microspores of the F_1 hybrids. Self-pollinated seed from dihaploid plants were analyzed for fatty acid analysis using methods described previously.

For chemical analysis, 10-seed bulk samples were hand ground with a glass rod in a 15-mL polypropylene tube and extracted in 1.2 mL 0.25 N KOH in 1:1 ether/methanol. The sample was vortexed for 30 sec. and heated for 60 sec. in a 60°C water bath. Four mL of saturated NaCl and 2.4 mL of iso-octane were added, and the mixture was vortexed again. After phase separation, 600 µL of the upper organic phase were pipetted into individual vials and stored under nitrogen at -5°C. One µL samples were injected into a Supelco SP-2330 fused silica capillary column (0.25 mm ID, 30 M length, 0.20 µm df).

The gas chromatograph was set at 180°C for 5.5 minutes, then programmed for a 2°C/minute increase to 212°C, and held at this temperature for 1.5 minutes. Total run time was 23 minutes. Chromatography settings were: Column head pressure - 15 psi, Column flow (He) - 0.7 mL/min., Auxiliary and Column flow - 33 mL/min., Hydrogen flow - 33 mL/min., Air flow - 400 mL/min., Injector temperature - 250°C, Detector temperature - 300°C, Split vent - 1/15.

Table 8 describes the upper and lower statistical thresholds for each fatty acid of interest.

TABLE 8

Statistical Thresholds for Specific Fatty Acids
Derived from Control Westar Plantings

Genotype	Percent Fatty Acids					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Sats*
M_3 Generation(1 in 10,000 rejection rate)						
Lower	3.3	1.4	--	13.2	5.3	6.0
Upper	4.3	2.5	71.0	21.6	9.9	8.3
M_4 Generation(1 in 800 rejection rate)						
Lower	3.6	0.8	--	12.2	3.2	5.3
Upper	6.3	3.1	76.0	32.4	9.9	11.2
M_5 Generation (1 in 755 rejection rate)						
Lower	2.7	0.9	--	9.6	2.6	4.5
Upper	5.7	2.7	80.3	26.7	9.6	10.0

*Sats=Total Saturate Content

EXAMPLE 2

High Oleic Acid Canola Lines

In the studies of Example 1, at the M_3 generation, 31 lines exceeded the upper statistical threshold for oleic acid ($\geq$ 71.0%). Line W7608.3 had 71.2% oleic acid. At the M_4 generation, its selfed progeny (W7608.3.5, since designated A129.5) continued to exceed the upper statistical threshold for $C_{18:1}$ with 78.8% oleic acid. M_5 seed of five self-pollinated plants of line A129.5 (ATCC 40811) averaged 75.0% oleic acid. A single plant selection, A129.5.3 had 75.6% oleic acid. The fatty acid composition of this high oleic acid mutant, which was stable under both field and greenhouse conditions to the M_7 generation, is summarized in Table 9. This line also stably maintained its mutant fatty acid composition to the M_7 generation in field trials in multiple locations. Over all locations the self-pollinated plants (A129) averaged 78.3% oleic acid. The fatty acid composition of the A129 for each Idaho trial location are summarized in Table 10. In multiple location replicated yield trials, A129 was not significantly different in yield from the parent cultivar Westar.

The canola oil of A129, after commercial processing, was found to have superior oxidative stability compared to Westar when measured by the Accelerated Oxygen Method (AOM), American Oil Chemists' Society Official Method Cd 12-57 for fat stability; Active Oxygen Method (revised 1989). The AOM of Westar was 18 AOM hours and for A129 was 30 AOM hours.

TABLE 9

Fatty Acid Composition of a High Oleic Acid Canola Line Produced by Seed Mutagenesis

	Percent Fatty Acids					
Genotype	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Sats
Westar	3.9	1.9	67.5	17.6	7.4	7.0
W7608.3 (M_3)	3.9	2.4	71.2	12.7	6.1	7.6
W7608.3.5 (M_4)	3.9	2.0	78.8	7.7	3.9	7.3
A129.5.3 (M_5)	3.8	2.3	75.6	9.5	4.9	7.6

Sats=Total Saturate Content

TABLE 10

Fatty Acid Composition of a Mutant High Oleic Acid Line at Different Field Locations in Idaho

	Percent Fatty Acids					
Location	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Sats
Burley	3.3	2.1	77.5	8.1	6.0	6.5
Tetonia	3.5	3.4	77.8	6.5	4.7	8.5
Lamont	3.4	1.9	77.8	7.4	6.5	6.3
Shelley	3.3	2.6	80.0	5.7	4.5	7.7

Sats=Total Saturate Content

The genetic relationship of the high oleic acid mutation A129 to other oleic desaturases was demonstrated in crosses made to commercial canola cultivars and a low linolenic acid mutation. A129 was crossed to the commercial cultivar Global ($C_{16:0}$ - 4.5%, $C_{18:0}$ - 1.5%, $C_{18:1}$ - 62.9%,$C_{18:2}$ - 20.0%, $C_{18:3}$ - 7.3%). Approximately 200 F_2 individuals were analyzed for fatty acid composition. The results are summarized in Table 11. The segregation fit 1:2:1 ratio suggesting a single co-dominant gene controlled the inheritance of the high oleic acid phenotype.

TABLE 11

Genetic Studies of A129 X Global

	$C_{18:1}$	Frequency	
Genotype	Content(%)	Observed	Expected
od-od-	77.3	43	47
od-od+	71.7	106	94
od+od+	66.1	49	47

A cross between A129 and IMC 01, a low linolenic acid variety ($C_{16:0}$ - 4.1%, $C_{18:0}$ - 1.9%, $C_{18:1}$ - 66.4%, $C_{18:2}$ - 18.1%, $C_{18:3}$ - 5.7%), was made to determine the inheritance of the oleic acid desaturase and linoleic acid desaturase. In the F_1 hybrids both the oleic acid and linoleic acid desaturase genes approached the mid-parent values indicating a co-dominant gene actions. Fatty acid analysis of the F_2 individuals confirmed a 1:2:1:2:4:2:1:2:1 segregation of two independent, co-dominant genes (Table 12). A line was selected from the cross of A129 and IMC01 and designated as IMC130 (ATCC deposit no. 75446) as described in U.S. Patent Application No. 08/425,108, incorporated herein by reference.

TABLE 12

Genetic Studies of A129 X IMC 01

Genotype	Ratio	Frequency Observed	Frequency Expected
od-od-ld-ld-	1	11	12
od-od-ld-ld+	2	30	24
od-od-ld+ld+	1	10	12
od-od+ld-ld-	2	25	24
od-od+ld-ld+	4	54	47
od-od+ld+ld+	2	18	24
od+od+ld-ld-	1	7	12
od+od+ld-ld+	2	25	24
od+od+ld+ld+	1	8	12

An additional high oleic acid line, designated A128.3, was also produced by the disclosed method. A 50-seed bulk analysis of this line showed the following fatty acid composition: $C_{16:0}$ - 3.5%, $C_{18:0}$ - 1.8%, $C_{18:1}$ - 77.3%, $C_{18:2}$ - 9.0%, $C_{18:3}$ - 5.6%, FDA Sats - 5.3%, Total Sats - 6.4%. This line also stably maintained its mutant fatty acid composition to the M_7 generation. In multiple locations replicated yield trials, A128 was not significantly different in yield from the parent cultivar Westar.

A129 was crossed to A128.3 for allelism studies. Fatty acid composition of the F_2 seed showed the two lines to be allelic. The mutational events in A129 and A128.3 although different in origin were in the same gene.

An additional high oleic acid line, designated M3028.-10 (ATCC 75026), was also produced by the disclosed method in Example 1. A 10-seed bulk analysis of this line showed the following fatty acid composition: $C_{16:0}$ - 3.5%, $C_{18:0}$ - 1.8%, $C_{18:1}$ - 77.3%, $C_{18:2}$ - 9.0%, $C_{18:3}$ - 5.6%, FDA Saturates - 5.3%, Total Saturates - 6.4%. In a single location replicated yield trial M3028.10 was not significantly different in yield from the parent cultivar Westar.

EXAMPLE 3

Low Linoleic Acid Canola

In the studies of Example 1, at the M_3 generation, 80 lines exceeded the lower statistical threshold for linoleic acid ($\leq$ 13.2%). Line W12638.8 had 9.4% linoleic acid. At the M_4 and M_5 generations, its selfed progenies [W12638.8, since designated A133.1 (ATCC 40812)] continued to exceed the statistical threshold for low $C_{18:2}$ with linoleic acid levels of 10.2% and 8.4%, respectively. The fatty acid composition of this low linoleic acid mutant, which was stable to the M_7 generation under both field and greenhouse conditions, is summarized in Table 13. In multiple location replicated yield trials, A133 was not significantly different in yield from the parent cultivar Westar. An additional low linoleic acid line, designated M3062.8 (ATCC 75025), was also produced by the disclosed method. A 10-seed bulk analysis of this line showed the following fatty acid composition: $C_{16:0}$ - 3.8%, $C_{18:0}$ - 2.3%, $C_{18:1}$ - 77.1%, $C_{18:2}$ - 8.9%, $C_{18:3}$ - 4.3%, FDA Sats-6.1%. This line has also stably maintained its mutant fatty acid composition in the field and greenhouse.

TABLE 13

Fatty Acid Composition of a Low Linoleic Acid Canola Line Produced by Seed Mutagenesis

	Percent Fatty Acids					
Genotype	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Sats[b]
Westar	3.9	1.9	67.5	17.6	7.4	7.0
W12638.8 (M_3)	3.9	2.3	75.0	9.4	6.1	7.5
W12638.8.1 (M_4)	4.1	1.7	74.6	10.2	5.9	7.1
A133.1.8 (M_5)	3.8	2.0	77.7	8.4	5.0	7.0

[a]Letter and numbers up to second decimal point indicate the plant line. Number after second decimal point indicates an individual plant.
[b]Sats=Total Saturate Content

EXAMPLE 4

Low Linolenic and Linoleic Acid Canola

In the studies of Example 1, at the M_3 generation, 57 lines exceeded the lower statistical threshold for linolenic acid ($\leq$ 5.3%). Line W14749.8 had 5.3% linolenic acid and 15.0% linoleic acid. At the M_4 and M_5 generations, its selfed progenies [W14749.8, since designated M3032 (ATCC 75021)] continued to exceed the statistical threshold for low $C_{18:3}$ with linolenic acid levels of 2.7% and 2.3%, respectively, and for a low sum of linolenic and linoleic acids with totals of 11.8% and 12.5% respectively. The fatty acid composition of this low linolenic acid plus linoleic acid mutant, which was stable to the M_5 generation under both field and greenhouse conditions, is summarized in Table 14. In a single location replicated yield trial M3032 was not significantly different in yield from the parent cultivar (Westar).

TABLE 14

Fatty Acid Composition of a Low Linolenic Acid Canola Line Produced by Seed Mutagenesis

Percent Fatty Acids

Genotype	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Sats
Westar	3.9	1.9	67.5	17.6	7.4	7.0
W14749.8 (M_3)	4.0	2.5	69.4	15.0	5.3	6.5
M3032.8 (M_4)	3.9	2.4	77.9	9.1	2.7	6.4
M3032.1 (M_5)	3.5	2.8	80.0	10.2	2.3	6.5

Sats=Total Saturate Content

EXAMPLE 5

Canola Lines Q508 and Q4275

Seeds of the *B. napus* line IMC-129 were mutagenized with methyl N-nitrosoguanidine (MNNG). The MNNG treatment consisted of three parts: pre-

soak, mutagen application, and wash. A 0.05M Sorenson's phosphate buffer was used to maintain pre-soak and mutagen treatment pH at 6.1. Two hundred seeds were treated at one time on filter paper (Whatman #3M) in a petri dish (100mm x 15mm). The seeds were pre-soaked in 15 mls of 0.05M Sorenson's buffer, pH 6.1, under continued agitation for two hours. At the end of the pre-soak period, the buffer was removed from the plate.

A 10mM concentration of MNNG in 0.05M Sorenson's buffer, pH 6.1, was prepared prior to use. Fifteen ml of 10m MNNG was added to the seeds in each plate. The seeds were incubated at 22°C±3°C in the dark under constant agitation for four (4) hours. At the end of the incubation period, the mutagen solution was removed.

The seeds were washed with three changes of distilled water at 10 minute intervals. The fourth wash was for thirty minutes. This treatment regime produced an LD60 population.

Treated seeds were planted in standard greenhouse potting soil and placed into an environmentally controlled greenhouse. The plants were grown under sixteen hours of light. At flowering, the racemes were bagged to produce selfed seed. At maturity, the M2 seed was harvested. Each M2 line was given an identifying number. The entire MNNG-treated seed population was designated as the Q series.

Harvested M2 seeds was planted in the greenhouse. The growth conditions were maintained as previously described. The racemes were bagged at flowering for selfing. At maturity, the selfed M3 seed was harvested and analyzed for fatty acid composition. For each M3 seed line, approximately 10-15 seeds were analyzed in bulk as described in Example 1.

High oleic-low linoleic M3 lines were selected from the M3 population using a cutoff of >82% oleic acid and <5.0% linoleic. From the first 1600 M3 lines screened for fatty acid composition, Q508 was identified. The Q508 M3 generation was advanced to the M4 generation in the greenhouse. Table 15 shows

the fatty acid composition of Q508 and IMC 129. The M4 selfed seed maintained the selected high oleic-low linoleic acid phenotype (Table 16).

TABLE 15

Fatty Acid Composition of A129 and High Oleic Acid M3 Mutant Q508

Line #	16:0	18:0	18:1	18:2	18:3
A129*	4.0	2.4	77.7	7.8	4.2
Q508	3.9	2.1	84.9	2.4	2.9

*Fatty acid composition of A129 is the average of 50 self-pollinated plants grown with the M3 population

M_4 generation Q508 plants had poor agronomic qualities in the field compared to Westar. Typical plants were slow growing relative to Westar, lacked early vegetative vigor, were short in stature, tended to be chlorotic and had short pods. The yield of Q508 was very low compared to Westar.

The M_4 generation Q508 plants in the greenhouse tended to be reduced in vigor compared to Westar. However, Q508 yields in the greenhouse were greater than Q508 yields in the field.

TABLE 16

Fatty Acid Composition of Seed Oil from Greenhouse-Grown Q508, IMC 129 and Westar.

Line	16:0	18:0	18:1	18:2	18:3	FDA Sats
IMC 129[a]	4.0	2.4	77.7	7.8	4.2	6.4
Westar[b]	3.9	1.9	67.5	17.6	7.4	>5.8
Q508[c]	3.9	2.1	84.9	2.4	2.9	6.0

[a]Average of 50 self-pollinated plants
[b]Data from Example 1
[c]Average of 50 self-pollinated plants

Nine other M4 high-oleic low-linoleic lines were also identified: Q3603, Q3733, Q4249, Q6284, Q6601, Q6761, Q7415, Q4275, and Q6676. Some of these lines had good agronomic characteristics and an elevated oleic acid level in seeds of about 80% to about 84%.

Q4275 was crossed to the variety Cyclone. After selfing for seven generations, mature seed was harvested from 93GS34-179, a progeny line of the Q4275xCyclone cross. Referring to Table 17, fatty acid composition of a bulk seed sample shows that 93GS34 retained the seed fatty acid composition of Q4275. 93GS34-179 also maintained agronomically desirable characteristics.

After more than seven generations of selfing of Q4275, plants of Q4275, IMC 129 and 93GS34 were field grown during the summer season. The selections were tested in 4 replicated plots (5 feet X 20 feet) in a randomized block design. Plants were open pollinated. No selfed seed was produced. Each plot was harvested at maturity, and a sample of the bulk harvested seed from each line was analyzed for fatty acid composition as described above. The fatty acid compositions of the selected lines are shown in Table 17.

TABLE 17

Fatty Acid Composition of
Field Grown IMC 129, Q4275 and 93GS34 Seeds

Line	**Fatty Acid Composition (%)**					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	FDA Sats
IMC 129	3.3	2.4	76.7	8.7	5.2	5.7
Q4275	3.7	3.1	82.1	4.0	3.5	6.8
93GS34-179	2.6	2.7	85.0	2.8	3.3	5.3

The results shown in Table 17 show that Q4275 maintained the selected high oleic - low linoleic acid phenotype under field conditions. The agronomic characteristics of Q4275 plants were superior to those of Q508.

M_4 generation Q508 plants were crossed to a dihaploid selection of Westar, with Westar serving as the female parent. The resulting F1 seed was

termed the 92EF population. About 126 F1 individuals that appeared to have better agronomic characteristics than the Q508 parent were selected for selfing. A portion of the F_2 seed from such individuals was replanted in the field. Each F2 plant was selfed and a portion of the resulting F3 seed was analyzed for fatty acid composition. The content of oleic acid in F_3 seed ranged from 59 to 79%. No high oleic (>80%) individuals were recovered with good agronomic type.

A portion of the F_2 seed of the 92EF population was planted in the greenhouse to analyze the genetics of the Q508 line. F_3 seed was analyzed from 380 F2 individuals. The $C_{18:1}$ levels of F_3 seed from the greenhouse experiment is depicted in Figure 1. The data were tested against the hypothesis that Q508 contains two mutant genes that are semi-dominant and additive: the original IMC 129 mutation as well as one additional mutation. The hypothesis also assumes that homozygous Q508 has greater than 85% oleic acid and homozygous Westar has 62-67% oleic acid. The possible genotypes at each gene in a cross of Q508 by Westar may be designated as:

AA = Westar Fad2^a

BB = Westar Fad2^b

aa = Q508 Fad2^{a-}

bb = Q508 Fad2^{b-}

Assuming independent segregation, a 1:4:6:4:1 ratio of phenotypes is expected. The phenotypes of heterozygous plants are assumed to be indistinguishable and, thus, the data were tested for fit to a 1:14:1 ratio of homozygous Westar: heterozygous plants: homozygous Q508.

Phenotypic Ratio	# of Westar Alleles	Genotype
1	4	AABB(Westar)
4	3	AABb,AaBB,AABb,AaBB
6	2	AaBb,AAbb,AaBb,AaBb,aaBB,AaBb
4	1	Aabb,aaBb,Aabb,aaBb
1	0	aabb (Q508)

Using Chi-square analysis, the oleic acid data fit a 1:14:1 ratio. It was concluded that Q508 differs from Westar by two major genes that are semi-dominant and additive and that segregate independently. By comparison, the genotype of IMC 129 is aaBB.

The fatty acid composition of representative F3 individuals having greater than 85% oleic acid in seed oil is shown in Table 18. The levels of saturated fatty acids are seen to be decreased in such plants, compared to Westar.

TABLE 18

92EF F_3 Individuals with >85% $C_{18:1}$ in Seed Oil

F3 Plant Identifier	Fatty Acid Composition (%)					
	C16:0	C18:0	C18:1	C18:2	C18:3	FDASA
+38068	3.401	1.582	85.452	2.134	3.615	4.983
+38156	3.388	1.379	85.434	2.143	3.701	4.767
+38171	3.588	1.511	85.289	2.367	3.425	5.099
+38181	3.75	1.16	85.312	2.968	3.819	4.977
+38182	3.529	0.985	85.905	2.614	3.926	4.56
+38191	3.364	1.039	85.737	2.869	4.039	4.459
+38196	3.557	1.182	85.054	2.962	4.252	4.739
+38202	3.554	1.105	86.091	2.651	3.721	4.713
+38220	3.093	1.16	86.421	1.931	3.514	4.314
+38236	3.308	1.349	85.425	2.37	3.605	4.718
+38408	3.617	1.607	85.34	2.33	3.562	5.224
+38427	3.494	1.454	85.924	2.206	3.289	4.948
+38533	3.64	1.319	85.962	2.715	3.516	4.959

EXAMPLE 6

Leaf and Root Fatty Acid Profiles of Canola Lines IMC-129, Q508, and Westar

Plants of Q508, IMC 129 and Westar were grown in the greenhouse. Mature leaves, primary expanding leaves, petioles and roots were harvested at the 6-8 leaf stage, frozen in liquid nitrogen and stored at -70°C. Lipid extracts were analyzed by GLC as described in Example 1. The fatty acid profile data are shown in Table 19. The data in Table 19 indicate that total leaf lipids in Q508 are higher in $C_{18:1}$ content than the $C_{18:2}$ plus $C_{18:3}$ content. The reverse is true for Westar and IMC 129. The difference in total leaf lipids between Q508 and IMC 129 is consistent with the hypothesis that a second Fad2 gene is mutated in Q508.

The $C_{16:3}$ content in the total lipid fraction was about the same for all three lines, suggesting that the plastid FadC gene product was not affected by the Q508 mutations. To confirm that the FadC gene was not mutated, chloroplast lipids were separated and analyzed. No changes in chloroplast $C_{16:1}$, $C_{16:2}$ or $C_{16:3}$ fatty acids were detected in the three lines. The similarity in plastid leaf lipids among Q508, Westar and IMC 129 is consistent with the hypothesis that the second mutation in Q508 affects a microsomal Fad2 gene and not a plastid FadC gene.

TABLE 19

	MATURE LEAF			EXPANDING LEAF			PETIOLE			ROOT		
	West.	129	3Q508	West.	129	3Q508	West.	129	3Q508	West.	129	3Q508
16:0	12.1	11.9	10.1	16.4	16.1	11.3	21.7	23.5	11.9	21.1	21.9	12.0
16:1	0.8	0.6	1.1		0.6	1.1	1.0	1.3	1.4	-	-	-
16:2	2.3	2.2	2.0	2.8	3.1	2.8	1.8	2.2	1.8	-	-	-
16:3	14.7	15.0	14.0	6.3	5.4	6.9	5.7	4.6	5.7	-	-	-
18:0	2.2	1.6	1.2	2.5	2.8	1.5	3.7	4.0	1.6	3.6	2.9	2.5
18:1	2.8	4.9	16.7	3.8	8.3	38.0	4.9	12.9	46.9	3.5	6.1	68.8
18:2	12.6	11.5	6.8	13.3	13.8	4.9	20.7	18.3	5.2	28.0	30.4	4.4
18:3	50.6	50.3	46.0	54.2	50.0	33.5	40.4	33.2	25.3	43.8	38.7	12.3

EXAMPLE 7

Sequences of Mutant and Wild-Type Delta-12 Fatty Acid Desaturases from *B. napus*

Primers specific for the FAD2 structural gene were used to clone the entire open reading frame (ORF) of the D and F delta-12 desaturase genes by reverse transcriptase polymerase chain reaction (RT-PCR). RNA from seeds of IMC 129, Q508 and Westar plants was isolated by standard methods and was used as template. The RT-amplified fragments were used for nucleotide sequence determination. The DNA sequence of each gene from each line was determined from both strands by standard dideoxy sequencing methods.

Sequence analysis revealed a G to A transversion at nucleotide 316 (from the translation initiation codon) of the D gene in both IMC 129 and Q508, compared to the sequence of Westar. The transversion changes the codon at this position from GAG to AAG and results in a non-conservative substitution of glutamic acid, an acidic residue, for lysine a basic residue. The presence of the same mutation in both lines was expected since the Q508 line was derived from IMC 129. The same base change was also detected in Q508 and IMC 129 when RNA from leaf tissue was used as template.

The G to A mutation at nucleotide 316 was confirmed by sequencing several independent clones containing fragments amplified directly from genomic DNA of IMC 129 and Westar. These results eliminated the possibility of a rare mutation introduced during reverse transcription and PCR in the RT-PCR protocol. It was concluded that the IMC 129 mutant is due to a single base transversion at nucleotide 316 in the coding region of the D gene of rapeseed microsomal delta 12-desaturase.

A single base transition from T to A at nucleotide 515 of the F gene was detected in Q508 compared to the Westar sequence. The mutation changes the codon at this position from CTC to CAC, resulting in the non-conservative substitution of a non-polar residue, leucine, for a polar residue, histidine, in the

resulting gene product. No mutations were found in the F gene sequence of IMC 129 compared to the F gene sequence of Westar.

These data support the conclusion that a mutation in a delta-12 desaturase gene sequence results in alterations in the fatty acid profile of plants containing such a mutated gene. Moreover, the data show that when a plant line or species contains two delta-12 desaturase loci, the fatty acid profile of an individual having two mutated loci differs from the fatty acid profile of an individual having one mutated locus.

The mutation in the D gene of IMC 129 and Q508 mapped to a region having a conserved amino acid motif (His-Xaa-Xaa-Xaa-His) found in cloned delta-12 and delta-15 membrane bound-desaturases (Table 20).

TABLE 20

Alignment of Amino Acid Sequences
of Cloned Canola Membrane Bound-Desaturases

Desaturase Gene	Sequence[a]	Position
Canola-fad2-D(mutant)	AHKCGH	109-114
Canola-Fad2-D	AHECGH	109-114
Canola-Fad2-F	AHECGH	109-114
Canola-FadC	GHDCAH	170-175
Canola-fad3 (mutant)	GHKCGH	94-99
Canola-Fad3	GHDCGH	94-99
Canola-FadD	GHDCGH	125-130

(FadD = Plastid delta 15, Fad3 = Microsomal delta-15),
(FadC = Plastid delta-12, Fad2 = Microsomal delta-12)

[a] One letter amino acid code; conservative substitutions are underlined; non-conservative substitutions are in bold.

EXAMPLE 8

Transcription and Translation of Microsomal Delta-12 Fatty Acid Desaturases

Transcription in vivo was analyzed by RT-PCR analysis of stage II and stage III developing seeds and leaf tissue. The primers used to specifically amplify delta-12 desaturase F gene RNA from the indicated tissues were sense primer 5'-GGATATGATGATGGTGAAAGA-3' and antisense primer 5'-TCTTTCACCATCATCATATCC-3'. The primers used to specifically amplify delta-12 desaturase D gene RNA from the indicated tissues were sense primer 5'-GTTATGAAGCAAAGAAGAAAC-3' and antisense primer 5'-GTTTCTTCTTTGCTTCATAAC-3'. The results indicated that mRNA of both the D and F gene was expressed in seed and leaf tissues of IMC 129, Q508 and wild type Westar plants.

In vitro transcription and translation analysis showed that a peptide of about 46 kD was made. This is the expected size of both the D gene product and the F gene product, based on sum of the deduced amino acid sequence of each gene and the cotranslational addition of a microsomal membrane peptide.

These results rule out the possibility that non-sense or frameshift mutations, resulting in a truncated polypeptide gene product, are present in either the mutant D gene or the mutant F gene. The data, in conjunction with the data of Example 7, support the conclusion that the mutations in Q508 and IMC 129 are in delta-12 fatty acid desaturase structural genes encoding desaturase enzymes, rather than in regulatory genes.

EXAMPLE 9

Development of Gene-Specific PCR Markers

Based on the single base change in the mutant D gene of IMC 129 described in above, two 5' PCR primers were designed. The nucleotide sequence of the primers differed only in the base (G for Westar and A for IMC 129) at the 3' end. The primers allow one to distinguish between mutant fad2-D and wild-type

Fad2-D alleles in a DNA-based PCR assay. Since there is only a single base difference in the 5' PCR primers, the PCR assay is very sensitive to the PCR conditions such as annealing temperature, cycle number, amount, and purity of DNA templates used. Assay conditions have been established that distinguish between the mutant gene and the wild type gene using genomic DNA from IMC 129 and wild type plants as templates. Conditions may be further optimized by varying PCR parameters, particularly with variable crude DNA samples. A PCR assay distinguishing the single base mutation in IMC 129 from the wild type gene along with fatty acid composition analysis provides a means to simplify segregation and selection analysis of genetic crosses involving plants having a delta-12 fatty acid desaturase mutation.

EXAMPLE 10

Transformation with Mutant and Wild Type Fad3 Genes

B. napus cultivar Westar was transformed with mutant and wild type Fad3 genes to demonstrate that the mutant Fad3 gene for canola cytoplasmic linoleic desaturase delta-15 desaturase is nonfunctional. Transformation and regeneration were performed using disarmed *Agrobacterium tumefaciens* essentially following the procedure described in WO 94/11516.

Two disarmed Agrobacterium strains were engineered, each containing a Ti plasmid having the appropriate gene linked to a seed-specific promoter and a corresponding termination sequence. The first plasmid, pIMC110, was prepared by inserting into a disarmed Ti vector the full length wild type Fad3 gene in sense orientation (nucleotides 208 to 1336 of SEQ ID 6 in WO 93/11245), flanked by a napin promoter sequence positioned 5' to the Fad3 gene and a napin termination sequence positioned 3' to the Fad3 gene. The rapeseed napin promoter is described in EP 0255378.

The second plasmid, pIMC205, was prepared by inserting a mutated Fad3 gene in sense orientation into a disarmed Ti vector. The mutant sequence contained mutations at nucleotides 411 and 413 of the microsomal Fad3 gene

described in WO93/11245, thus changing the sequence for codon 96 from GAC to AAG. The amino acid at codon 96 of the gene product was thereby changed from aspartic acid to lysine. See Table 20. A bean (*Phaseolus vulgaris*) phaseolin (7S seed storage protein) promoter fragment of 495 base pairs was placed 5' to the mutant Fad3 gene and a phaseolin termination sequence was placed 3' to the mutant Fad3 gene. The phaseolin sequence is described in Doyle et al., (1986) J. Biol. Chem. 261:9228-9238) and Slightom et al., (1983) Proc. Natl. Acad. Sci. USA 80:1897-1901.

The appropriate plasmids were engineered and transferred separately to *Agrobacterium* strain LBA4404. Each engineered strain was used to infect 5 mm segments of hypocotyl explants from Westar seeds by cocultivation. Infected hypocotyls were transferred to callus medium and, subsequently, to regeneration medium. Once discernable stems formed from the callus, shoots were excised and transferred to elongation medium. The elongated shoots were cut, dipped in Rootone™, rooted on an agar medium and transplanted to potting soil to obtain fertile T1 plants. T2 seeds were obtained by selfing the resulting T1 plants.

Fatty acid analysis of T2 seeds was carried out as described above. The results are summarized in Table 21. Of the 40 transformants obtained using the pIMC110 plasmid, 17 plants demonstrated wild type fatty acid profiles and 16 demonstrated overexpression. A proportion of the transformants are expected to display an overexpression phenotype when a functioning gene is transformed in sense orientation into plants.

Of the 307 transformed plants having the pIMC205 gene, none exhibited a fatty acid composition indicative of overexpression. This result indicates that the mutant fad3 gene product is non-functional, since some of the transformants would have exhibited an overexpression phenotype if the gene product were functional.

TABLE 21

Overexpression and Co-suppression Events in
Westar Populations Transformed with pIMC205 or pIMC110.

Construct	Number of Transformants	α-Linolenic Acid Range(%)	Overexpression Events (>10% linolenic)	Co-Suppression Events (<4.0% linolenic)	Wild Type Events
pIMC110	40	2.4 - 20.6	16	7	17
pIMC205	307	4.6 - 10.4	0	0	307

Fatty acid compositions of representative transformed plants are presented in Table 22. Lines 652-09 and 663-40 are representative of plants containing pIMC110 and exhibiting an overexpression and a co-suppression phenotype, respectively. Line 205-284 is representative of plants containing pIMC205 and having the mutant fad3 gene.

TABLE 22

Fatty Acid Composition of T2 Seed
From Westar Transformed With pIMC205 or pIMC110.

Line	Fatty Acid Composition (%)				
	C16:0	C18:0	C18:1	C18:2	C18:3
652-09 pIMC110 overexpression	4.7	3.3	65.6	8.1	14.8
663-40 pIMC110 co-suppression	4.9	2.1	62.5	23.2	3.6
205-284 pIMC205	3.7	1.8	68.8	15.9	6.7

EXAMPLE 11

Sequences of Wild Type and Mutant Fad2-D and Fad2-F

High molecular weight genomic DNA was isolated from leaves of Q4275 plants (Example 5). This DNA was used as template for amplification of Fad2-D and Fad2-F genes by polymerase chain reaction (PCR). PCR amplifications were carried out in a total volume of 100 µl and contained 0.3 µg genomic DNA, 200 µM deoxyribonucleoside triphosphates, 3 mM $MgSO_4$, 1-2 Units DNA polymerase and 1X Buffer (supplied by the DNA polymerase manufacturer). Cycle conditions were: 1 cycle for 1 min at 95°C, followed by 30 cycles of 1 min at 94°C, 2 min at 55°C and 3 min at 73°C.

The Fad2-D gene was amplified once using Elongase® (Gibco-BRL). PCR primers were: CAUCAUCAUCAUCTTCTTCGTAGGGTTCATCG and

CUACUACUACUATCATAGAAGAGAAAGGTTCAG for the 5' and 3' ends of the gene, respectively.

The Fad2-F gene was independently amplified 4 times, twice with Elongase® and twice with Taq polymerase (Boehringer Mannheim). The PCR primers used were: 5'CAUCAUCAUCAUCATGGGTGCACGTGGAAGAA3' and 5'CUACUACUACUATCTTTCACCATCATCATATCC3' for the 5' and 3' ends of the gene, respectively.

Amplified DNA products were resolved on an agarose gel, purified by JetSorb® and then annealed into pAMP1 (Gibco-BRL) via the $(CAU)_4$ and $(CUA)_4$ sequences at the ends of the primers, and transformed into *E. coli* DH5α.

The Fad2-D and Fad2-F inserts were sequenced on both strands with an ABI PRISM 310 automated sequencer (Perkin-Elmer) following the manufacturer's directions, using synthetic primers, AmpliTaq® DNA polymerase and dye terminator.

The Fad2-D gene was found to have an intron upstream of the ATG start codon. As expected, the coding sequence of the gene contained a G to A mutation at nucleotide 316, derived from IMC 129 (Fig. 2).

A single base transversion from G to A at nucleotide 908 was detected in the F gene sequence of the Q4275 amplified products, compared to the wild type F gene sequence (Fig. 2). This mutation changes the codon at amino acid 303 from GGA to GAA, resulting in the non-conservative substitution of a glutamic acid residue for a glycine residue (Table 3 and Fig. 3). Expression of the mutant Q4275 Fad2-F delta-12 desaturase gene in plants alters the fatty acid composition, as described hereinabove.

EXAMPLE 12

High Erucic, High Oleic Acid Rapeseed

The breeding procedure designed to produce novel fatty acid compositions in rapeseed is outlined in Figure 4. In general, crosses were made between a high erucic acid line and a high oleic acid line. The high erucic acid

line, designated HECO1 (sold under the trade name Hero), contains about 45.5% erucic acid (Table 23). The high oleic acid lines were designated 93GS66A-130 and 93GS34A-179 and were derived from 93GS. See, for example, Example 5 and Table 17. These lines contain about 84% oleic acid in their seed oil (Table 24).

TABLE 23

Fatty Acid Composition of HEC01

Fatty Acid	Weight (%)
$C_{14:0}$	0.05
$C_{16:0}$	3.60
$C_{16:1}$	0.36
$C_{16:0}$	1.66
$C_{16:1}$	14.72
$C_{18:2}$	10.67
$C_{18:3}$	9.71
$C_{20:0}$	1.36
$C_{20:1}$	9.04
$C_{20:2}$	0.48
$C_{22:0}$	1.74
$C_{22:1}$	45.45
$C_{24:0}$	0.49
$C_{24:1}$	0.81

TABLE 24
Fatty Acid Composition of 93GS66A-130 and 93GS34A-179

Fatty Acid	Weight(%) of 93GS66A-130	Weight(%) of 93GS34A-179
$C_{14:0}$	0.04	0.05
$C_{16:0}$	3.25	3.23
$C_{16:1}$	0.25	0.25
$C_{18:0}$	1.60	1.94
$C_{18:1}$	84.38	83.71
$C_{18:2}$	2.58	3.14
$C_{18:3}$	4.86	4.76
$C_{20:0}$	0.56	0.65
$C_{20:1}$	1.57	1.41
$C_{20:2}$	0.05	0.04
$C_{22:0}$	0.37	0.39
$C_{22:1}$	0.06	0.03
$C_{24:0}$	0.20	0.18
$C_{24:1}$	0.21	0.18

The F_1 generations of crosses between HEC01 x 93GS66A-130, and HEC01 x 93GS34A-179, were designated 96.801 and 96.804, respectively. F_1 96.801 and 96.804 plants were self-pollinated to produce F_2 seed. Overall, 622 random single F_2 seeds were analyzed for their fatty acid composition. Table 25 summarizes the average percent and standard deviation for total monounsaturated content, oleic acid, eicosenoic acid, erucic acid, total polyunsaturated and total saturated fatty acid content of these 622 seeds.

TABLE 25

Fatty acid	%
Total long chain monounsaturated	78.90 ± 4.07
Palmitoleate	0.28 ± 0.06
Oleic Acid	45.33 ± 9.91
Eicosenoic Acid	14.84 ± 2.84
Erucic Acid	17.97 ± 8.9
Nervonic Acid	0.48 ± 0.21
Total polyunsaturated	7.10 ± 1.05
Total saturated	13.99 ± 3.83

Analysis of this data indicate that the frequency distributions deviate from a normal distribution. The total long chain monounsaturated content frequency distribution is slightly skewed to the right (-0.0513), and the eicosenoic acid content distribution is strongly skewed to the right (-1.715). Frequency distributions for oleic acid and erucic acid content are strongly skewed to the left (0.397 and 0.177, respectively). Skewness was calculated using Lotus 1-2-3 for Windows (release 5.0).

Table 26 describes characteristics of selected populations within the total population of seeds. For example, 151 seeds had a long chain monounsaturated fatty acid content greater than 82% (Table 26, column B). Within this population, the average oleic, eicosenoic and erucic acid content was about 48%, 16%, and 19%, respectively. Total polyunsaturated fatty acid content (C18:2, C18:3, and C20:2) was about 9% and total saturated fatty acid content was less than 7%.

Forty-seven of the 622 seeds had a long chain monounsaturated content greater than 85% (Table 26, column C). The average oleic, eicosenoic and erucic acid content within these seeds was 51%, 17%, and 17%, respectively. Total saturated and total polyunsaturated fatty acids were each less than 7%.

Twenty-three of the seeds had an eicosenoic acid content greater than 19% (Table 26, column F). Within these seeds, the average oleic acid erucic acid content was about 44% and 19%, respectively. Total polyunsaturated fatty acids were less than 10% and total saturated fatty acids were less than 7%.

Table 26

	A	B	C	D	E	F
Total Saturated	6.76 ± 0.72	6.65 ± 0.07	6.68 ± 0.61	6.85 ± 0.98	6.85 ± 0.93	6.66 ± 0.78
C14:0	0.04 ± 0.04	0.07 ± 0.05	0.06 ± 0.03	0.07 ± 0.04	0.06 ± 0.64	0.07 ± 0.05
C16:0	3.42 ± 0.37	3.35 ± 0.34	3.28 ± 0.32	3.45 ± 0.40	3.51 ± 0.42	3.31 ± 0.33
C18:0	1.92 ± 0.33	1.93 ± 0.32	2.06 ± 0.32	1.83 ± 0.30	1.90 ± 0.30	1.93 ± 0.23
C20:0	0.77 ± 0.14	0.76 ± 0.14	0.76 ± 0.13	0.80 ± 0.13	0.76 ± 0.12	0.79 ± 0.16
C22:0	0.38 ± 0.14	0.36 ± 0.12	0.37 ± 0.11	0.42 ± 0.15	0.35 ± 0.12	0.35 ± 0.29
C24:0	0.21 ± 0.19	0.19 ± 0.14	0.20 ± 0.17	0.28 ± 0.76	0.26 ± 0.71	0.19 ± 0.24
Total Monounsaturated	82.91 ± 2.11	84.21 ± 1.64	86.21 ± 1.00	79.49 ± 4.00	80.36 ± 3.75	83.92 ± 2.43
C16:1	0.28 ± 0.05	0.27 ± 0.05	0.27 ± 0.04	0.27 ± 0.05	0.27 ± 0.05	0.26 ± 0.05
C18:1	46.45 ± 9.47	47.66 ± 9.22	51.33 ± 8.96	39.29 ± 6.21	43.55 ± 6.91	44.08 ± 2.89
C20:1	16.91 ± 2.57	16.41 ± 2.47	16.72 ± 2.40	15.56 ± 2.22	16.78 ± 1.30	19.97 ± 0.63
C22:1	19.69 ± 8.45	19.38 ± 8.25	17.39 ± 12.26	23.81 ± 5.89	19.27 ± 6.39	19.09 ± 2.32
C24:1	0.49 ± 0.19	0.48 ± 0.17	0.50 ± 0.18	0.56 ± 0.19	0.49 ± 0.21	0.52 ± 0.29
Total Polyunsaturated	10.33 ± 2.10	9.14 ± 1.71	7.11 ± 0.98	13.66 ± 3.87	12.80 ± 3.60	9.43 ± 2.43
C18:2	5.16 ± 1.5	4.36 ± 1.24	3.17 ± 0.82	7.25 ± 2.62	6.58 ± 2.40	3.96 ± 1.39
C18:3	5.02 ± 1.18	4.65 ± 1.07	3.84 ± 0.69	6.19 ± 1.61	6.02 ± 1.52	5.27 ± 1.25
C20:2	0.15 ± 0.10	0.13 ± 0.04	0.10 ± 0.04	0.22 ± 0.11	0.19 ± 0.10	0.20 ± 0.28

A=>80% total long chain monounsaturated content, n=247; B=>82% total long chain monounsaturated content, n=151; C=>85% total long chain monounsaturated content, n=47; D=>15% erucic acid, n=318; E=>15% eicosenoic acid, n=323; F=>19% eicosenoic acid, n=23

Fatty acid composition of selected single seeds is presented in Table 27. V800655.334 was a single seed that had a long chain monounsaturated fatty acid content of approximately 84%. The oleic acid, eicosenoic acid and erucic acid content was 33.48%, 17.14%, and 32.23%, respectively. The total polyunsaturated fatty acid content was approximately 10%. The linoleic, α-linolenic and erucic acid content was 3.54%, 6.01%, and 0.15%, respectively.

V800655.126 was a single seed that had a long chain monounsaturated fatty acid content of approximately 85% (42.67% oleic acid, 16.21% eicosenoic acid, and 25.37% erucic acid). The total polyunsaturated fatty acid content was approximately 8% (4.87% linoleic acid, 3.05% α-linolenic acid, and 0.13% eicosadienoic acid).

V800654.9 was a single seed that had a long chain monounsaturated fatty acid content of 89% (51.53% oleic acid, 16.94% eicosenoic acid, and 19.24% erucic acid). The total polyunsaturated fatty acid content was approximately 8% (4.87% linoleic acid, 3.05% α-linolenic acid, and 0.13% eicosadienoic acid).

Single seeds having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% were planted in a greenhouse, grown to maturity and self-pollinated. Seed (F_3 generation) from each plant were harvested. A bulk seed sample from each F_2 plant is analyzed for fatty acid composition.

TABLE 27

Fatty Acid Composition of Selected Single Seeds

Fatty Acid	V800655.334 Weight (%)	V800655.126 Weight (%)	V800654.9 Weight (%)
$C_{14:0}$	0.07	0.05	0.03
$C_{16:0}$	3.49	3.52	2.98
$C_{16:1}$	0.34	0.28	0.28
$C_{18:0}$	1.64	1.89	1.65
$C_{18:1}$	33.48	42.67	51.53
$C_{18:2}$	3.54	4.87	2.09
$C_{18:3}$	6.01	3.05	3.53
$C_{20:0}$	0.86	0.87	0.68
$C_{20:1}$	17.14	16.21	16.94
$C_{20:2}$	0.15	0.13	0.14
$C_{22:0}$	0.41	0.35	0.24
$C_{22:1}$	32.23	25.37	19.24
$C_{24:0}$	0.12	0.13	0.14
$C_{24:1}$	0.52	0.61	0.59

EXAMPLE 13

Additional crosses were made between Hero and several high oleic lines (Table 28) to increase the seed erucic acid content through a reduction in polyunsaturates content and increase in total monunsaturates content. The high oleic acid lines included 048X058 and Q4275X663-40. The 048X058 line resulted from a cross of two separate transformed lines. The 048X058 line contains a co-suppression event resulting from introduction of the 663-40 transgene described above, and a second co-suppression event resulting from a transgene that includes an oleosin promoter linked to an oleic desaturase gene. The Q4275X663-40 line was derived from a cross of Q4275 (Example 5 and Table 17) by 663-40. The

663-40 line contains a co-suppression event resulting from a transgene that includes a napin promotor linked to a linoleic desaturase gene. Plants of each line were grown in growth chambers under 16 hrs of light at 23/17°C day/night temperature. Flowers were emasculated prior to opening and covered to prevent cross pollination. On the following day, stigmas of emasculated flowers were pollinated with the desired pollen donor. At pod maturity the F1 seed was harvested.

TABLE 28

Crossing block

Cross Number	Female Parent	Female Phenotype	Male Parent	Male Phenotype	Source of Male Phenotype
HEHOA	HEC101	High 22:1	048X058	High 18:1/ Low 18:3	Transgenes
HEHOB	HEC101	High 22:1	Q4275X663-40	High 18:1/ Low 18:3	Mutant/ Transgene
HEHOC	HEC101	High 22:1	Q4275X663-40	High 18:1/ Low 18:3	Mutant/ Transgene

F1 seed generated from the crosses in Table 28 were advanced to F2 seed generation in the growth chamber. Ten seeds were individually planted for each cross. At flowering the plants were covered with bags to ensure self pollination. The F2 seeds were harvested at maturity.

The seeds were germinated on filter paper at room temperature in the dark. Eighteen to 24 hours after the start of germination, one cotyledon was removed from the seed for extraction of fatty acids. Fatty acid compositions were determined using gas chromatography. Selected F2 half seeds having a high erucic content are shown in Tables 29 and 30.

F_2 half seeds were planted in soil and grown under growth chamber conditions described above. At flowering the plants were covered with bags for self pollination. After maturity, F_3 seed was harvested and analyzed for fatty acid composition. Seeds were analyzed using a 10-15 seed sample size. The results of the analysis are in Tables 31 and 32.

TABLE 29

Fatty Acid Composition of F_2 Half Seeds of HEHOA [HE101X(048X052)]

Sample No.	Fatty Acid Composition (%)										
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0	C24:1
VL10186-1	3.34	1.83	49.7	3.32	1.59	0.87	19.62	0.30	18.10	0.39	0.35
VL10186-5	2.61	1.07	29.14	5.81	2.42	0.71	14.99	0.31	40.90	0.93	0.60
VL10186-33	3.47	1.32	29.73	4.38	2.98	0.86	12.22	0.44	41.21	1.50	1.28
VL10186-59	4.01	1.68	41.38	2.96	1.69	1.08	19.72	0.43	26.05	0	0.55
VL10186-67	3.90	1.29	29.10	3.65	2.89	0.88	13.79	0.52	40.96	1.31	1.09
VL10186-74	2.76	1.25	34.04	2.63	1.45	0.75	16.64	0.38	38.67	0.14	0.95
VL10186-88	3.23	1.39	48.97	3.27	1.50	0.60	19.71	0.17	20.48	0	0.36

TABLE 30

Fatty Acid Compositions of Half Seeds of HEHOC [HE101X(Q4275X663-40)]

Sample No.	Fatty Acid Composition (%)										
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0	C24:1
VL10200-214	2.24	0.74	31.66	3.01	6.24	0.46	11.79	0.41	40.60	0.86	1.57
VL10200-231	3.89	1.03	31.51	12.50	2.41	0.54	14.17	0.29	32.23	0	0.75
VL10200-238	3.36	0.95	33.19	8.99	1.66	0.55	14.35	0.21	33.61	0.83	1.02
VL10200-267	3.12	1.02	30.18	7.61	1.52	0.59	14.53	0.19	39.41	0.24	1.013
VL10203-50	2.63	0.97	31.79	8.47	1.99	0.58	14.58	0.25	37.41	0.13	0.59
VL10200-293	2.71	0.78	32.83	6.85	1.88	0.46	13.11	0.32	39.18	0.82	0.67

TABLE 31

Fatty acid composition of F3 lines of 97HEHOA [HE101X(048X052)]

Sample No.	Fatty Acid Composition (%)										
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0	C24:1
HEHOA-74	2.51	1.03	27.44	3.72	3.55	0.65	13.60	0.30	45.57	0.14	1.03
HEHOA-01	3.66	1.40	47.79	6.47	2.97	0.62	19.23	0.25	16.11	0.14	0.76
HEHOA-67	2.47	0.84	20.43	7.25	3.89	0.69	10.33	0.47	52.09	0.16	0.86
HEHOA-59	2.81	1.08	27.01	7.88	2.82	0.69	16.15	0.32	39.68	0.15	0.86
HEHOA-88	3.16	1.21	44.85	9.21	2.31	0.49	16.30	0.21	21.00	0.12	0.58
HEHOA-33	2.53	0.79	21.90	9.52	3.55	0.53	11.51	0.31	47.59	0.13	1.08
HEHOA-5	2.93	1.01	23.67	10.26	2.00	0.63	14.34	0.38	42.98	0.15	1.06

TABLE 32

Fatty acid composition of F3 lines of HEHOC [HE101X(Q4275X663-40)]

Sample No.	Fatty Acid Composition (%)										
	C16:0	C16:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0	C24:1
HEHOC-214	2.47	1.12	31.15	3.77	3.84	0.77	13.78	0.43	41.15	0.17	0.97
HEHOC-267	2.62	1.42	31.64	6.44	1.30	0.84	15.64	0.39	38.15	0.16	0.95
HEHOC-293	2.73	1.13	32.08	7.23	2.18	0.72	14.88	0.41	37.17	0.17	0.81
HEHOC-238	2.90	1.05	35.20	9.37	1.76	0.66	14.88	0.38	32.05	0.1	1.01
HEHOC(2)-50	2.60	0.93	31.16	5.66	2.09	0.61	14.93	0.31	40.30	0.11	0.88
HEHOC(2)-156	3.19	1.71	46.56	3.05	1.59	0.94	16.41	0.40	24.67	0.19	0.83

HEHOC-214 F_3 seeds were planted and grown under growth chamber conditions and self-pollinated as described above. At maturity, F_4 seeds were harvested and analyzed for fatty acid composition, using bulk samples of 10-15 seeds. The seed fatty acid composition of three F_4 lines is shown in Table 33. All three samples had a long chain monounsaturated fatty acid content of greater than 82% and an erucic acid content of greater than 37% based on total fatty acid composition. Genes affecting fatty acid composition are still segregating in this F_4 generation material. Selection in subsequent generations will fix the genetic makeup and result in lines having a seed fatty acid composition of about 25-30% oleic acid, about 3-4% linoleic acid, about 1-2% α-linolenic acid, about 45-50% erucic acid and about 10-13% eicosenoic acid.

TABLE 33

Fatty acid composition of F4 lines of HEHOC 214

Sample No.	Fatty Acid Composition (%)											
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0	C24:1	Total Sats
1	1.91	1.33	35.9	3.17	1.21	0.97	16.57	0.52	37.1	0.23	0.76	4.9
2	1.86	0.87	24.26	8.49	1.01	0.7	12.41	0.4	48.56	0.18	0.90	4.0
3	1.94	0.83	26.67	8.31	1.02	0.64	14.17	0.34	44.71	0.14	0.82	3.9

EXAMPLE 14

Seeds from the lines of Table 31 were planted in two separate plots. Plants in each plot were allowed to open-pollinate. Oil was extracted from seeds produced on each plot; the fatty acid compositions of each oil are shown in Table 34. A sample of each oil was refined and bleached (high long chain monounsaturate plot 1) or refined, bleached, and deodorized (high long chain monounsaturate plot 2). Oil from plot 1 had a total saturated fatty acid content of 5.55%, a long chain monounsaturated fatty acid content of 85.23%, and a total polyunsaturated fatty acid content of 6.80%. Oil from plot 2 had a total saturated fatty acid content of 5.22%, a long chain monounsaturated fatty acid content of 82.90%, and a total polyunsaturated fatty acid content of 9.56%. The iodine values of oil from plots 1 and 2 were 79 and 81.7, respectively. Oil from plot 1, which was not deodorized, contained 420 ppm of tocopherol. Oil from plot 2 contained 280 ppm of tocopherol. Oil from plots 1 and 2 had average oxidative stabilities of 70 AOM hours (n=2, 69 and 71 AOM hours) and 49.5 AOM hours (n=2, 48 and 51 AOM hours), respectively.

TABLE 34

Fatty Acid Composition of High Long Chain Monounsaturate Rapeseed Oil

Plot No.	Fatty Acid Composition (%)								
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1
Plot 1	2.45	1.50	31.50	3.68	2.45	0.90	13.20	0.39	39.3
Plot 2	2.55	1.30	29.70	6.35	2.46	0.76	12.50	0.36	39.50

Table 35 provides characteristics of oils from plots 1 and 2, which were determined by differential scanning calorimetry using a Perkin Elmer Model 7 differential scanning calorimeter. Samples of 7-12 mg were placed in sample pans, sealed, and loaded into an autosampler. Samples were cooled from an initial temperature of 20°C, which was maintained for 1 minute, to -30°C at a rate of

40°C per minute. Samples were held at -30°C for 10 minutes, then heated to 75°C at a rate of 5°C per minute to obtain a melting curve. Samples were held at 75°C for 10 minutes, then cooled to -30°C at a rate of 5°C per minute to obtain a cooling curve. The results show that these oils, having a long chain monounsaturate content of greater than 82% and an erucic acid content of greater than 15%, exhibit melting points of about 2-3° C. By comparison, trierucin is a solid at room temperature.

TABLE 35

Characteristics of High Long Chain Monounsaturate Rapeseed Oil

Plot	MP (°C)	Onset of Crystallization (°C)	ΔH (j/g)
1	3.2	-24	88.5
2	2.2	-27	92.6

EXAMPLE 15

Seeds of *Brassica napus* variety IMC 129 were mutagenized with MNNG as described in Example 5. Treated seeds were grown as described in Example 5 and selection for decreased seed stearic acid or decreased palmitic acid content was carried out at the M3 generation. Plants from two selected lines, designated ZW1441 (decreased palmitic acid) and Y30137 (decreased stearic acid), were crossed with HE101. ZW1441XHE101 progeny that produced seeds having decreased palmitic acid and elevated erucic acid were selected. Y30137XHE101 progeny that produced seeds having decreased stearic acid and elevated erucic acid were selected. The fatty acid composition of seeds from representative F_4 generation selected lines is shown in Table 36. The results show that seeds having a long chain monounsaturate content of about 82% or greater, an erucic acid content of 15% or greater and a total saturates content of less than 4% (e.g., about 2.0 to about 4.0%) can be achieved.

TABLE 36
Fatty Acid Composition of F_4 Lines

Sample No.	Fatty Acid Composition (%)								
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1
ZW1441	2.73	1.74	73.46	11.46	7.52	0.67	1.47	0.34	0
ZW1441 x HE101									
1	1.99	0.97	14.89	10.66	6.77	0.97	8.57	0.78	52.4
2	2.15	0.90	14.89	12.09	7.01	0.80	8.84	0.58	50.7
3	2.18	1.02	13.86	11.24	6.64	0.97	8.89	0.65	52.33
Y30137	3.44	1.14	78.08	7.41	6.23	0.71	1.70	0.46	0.04
HE101 x Y30137									
1	2.26	0.77	17.17	9.31	6.32	0.69	10.44	0.50	50.99
2	1.99	0.75	26.42	8.48	7.47	0.58	12.97	0.39	39.5
3	1.99	0.79	24.62	5.22	6.32	0.64	13.84	0.35	44.86

To the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various specific embodiments herein described and illustrated may be further modified to incorporate features shown in other of the specific embodiments. All patents, publications, and other references cited herein are incorporated by reference in their entirety.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those skilled in the art without deviating from the spirit and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A *Brassica* plant producing seeds having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition.

2. The plant of claim 1, said seeds having an oleic acid content of at least about 37% based on total fatty acid composition.

3. The plant of claim 1, said seeds having an eicosenoic acid content of at least about 14% based on total fatty acid composition.

4. The plant of claim 1, wherein said monounsaturated fatty acid content is from about 85% to about 90%.

5. The plant of claim 4, said seeds having an oleic acid content of at least about 42% based on total fatty acid composition.

6. The plant of claim 5, wherein said oleic acid content is from about 47% to about 56%.

7. The plant of claim 4, said seeds having an erucic acid content of from about 17% to about 31% based on total fatty acid composition.

8. The plant of claim 4, said seeds having an eicosenoic acid content from about 15% to about 21% based on total fatty acid composition.

9. The plant of claim 1, said seeds having a saturated fatty acid content of less than about 7% based on total fatty acid composition.

10. The plant of claim 9, said seeds having a saturated fatty acid acid content of less than about 4% based on total fatty acid composition.

11. The plant of claim 10, said seeds having a saturated fatty acid acid content of about 2% to about 4% based on total fatty acid composition.

12. The plant of claim 1, said seeds having a polyunsaturated fatty acid content of less than about 11% based on total fatty acid composition.

13. The plant of claim 12, said seeds having a polyunsaturated fatty acid content of about 6% to about 11% based on total fatty acid composition.

14. The plant of claim 1, said seeds having an α-linolenic acid content of about 1% to about 2% based on total fatty acid composition.

15. Progeny of the plant of claim 1, said progeny having said long chain monounsaturated fatty acid content and said erucic acid content.

16. A *Brassica* seed oil having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition.

17. The oil of claim 16, said oil having an oleic acid content of at least about 37% based on total fatty acid composition.

18. The oil of claim 16, said oil having an eicosenoic acid content of at least about 14% based on total fatty acid composition.

19. The oil of claim 16, wherein said monounsaturated fatty acid content is from about 85% to about 90%.

20. The oil of claim 19, said oil having an oleic acid content of at least about 42% based on total fatty acid composition.

21. The oil of claim 20, wherein said oleic acid content is from about 47% to about 56%.

22. The oil of claim 19, said oil having an erucic acid content of from about 17% to about 31% based on total fatty acid composition.

23. The oil of claim 19, said oil having an eicosenoic acid content from about 15% to about 21% based on total fatty acid composition.

24. The oil of claim 16, said oil having a saturated fatty acid content of less than about 7% based on total fatty acid composition.

25. The oil of claim 16, said oil having a polyunsaturated fatty acid content of less than about 11% based on total fatty acid composition.

26. The oil of claim 25, wherein said polyunsaturated fatty acid content is less than about 9%.

27. A *Brassica* seed oil having a long chain monounsaturated fatty acid content of at least about 82%, wherein the sum of the nervonic acid, erucic acid and eicosenoic acid content is from about 50% to about 66% based on total fatty acid composition.

28. The seed oil of claim 27, wherein the oleic acid content is from about 25% to about 30%.

29. A method of producing a *Brassica* plant, said method comprising the steps of crossing a first plant line with a second plant line and selecting progeny of said cross, wherein said first plant line has an erucic acid content of at least about 45% based on total fatty acid composition and said second plant line has an oleic acid content of at least about 84% based on total fatty acid composition, said progeny having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition.

30. A method of making a vegetable oil, said method comprising the steps of crushing *Brassica* seeds having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition, and extracting said vegetable oil from said crushed seeds.

31. The method of claim 30, further comprising the steps of refining and bleaching said oil.

32. The method of claim 31, further comprising the step of deodorizing said oil.

33. A lubricant, comprising a *Brassica* oil having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition, and an additive.

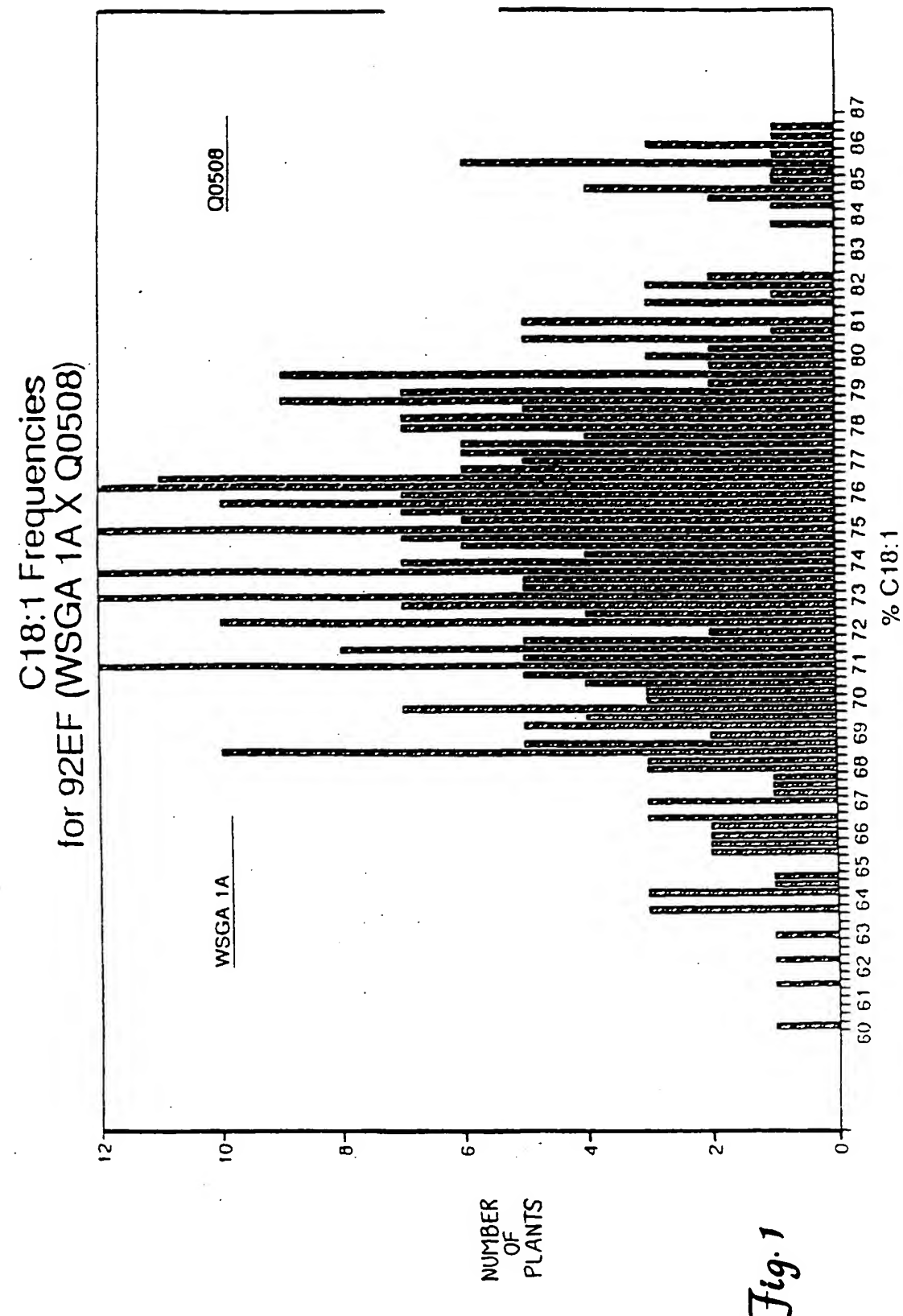
34. The lubricant of claim 33, wherein said additive is selected from the group consisting of an antioxidant, a rust inhibitor, a corrosion inhibitor, a pour point depressant, an anti-foam additive, a colorant and a detergent.

35. The lubricant of claim 33, wherein said additive is present in an amount from about 0.01% to about 20% by weight.

36. A hydraulic fluid comprising a *Brassica* oil having a long chain monounsaturated fatty acid content of at least about 82% and an erucic acid content of at least about 15% based on total fatty acid composition, and an additive.

37. The hydraulic fluid of claim 36, wherein said additive is selected from the group consisting of an antioxidant, a rust inhibitor, a corrosion inhibitor, a pour point depressant, an anti-foam additive, a colorant and a detergent.

38. The hydraulic fluid of claim 36, wherein said additive is present in an amount from about 0.01% to about 20% by weight.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for 92EF (WSGA 1A X Q0508)
Q0508
WSGA 1A
NUMBER OF PLANTS
% C18:1
0
2
4
6
8
10
12
60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87

Fig. 1

```
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1   ATGGGTGCAGGTGGAAGAATGCAAGTGTCTCCTCCCTCCA Fad2-F (GA908) Q4275

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41  AGAAGTCTGAAACCGACACCATCAAGCGCGTACCCTGCGA Fad2-F wt
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41  AGAAGTCTGAAACCGACACCATCAAGCGCGTACCCTGCGA Fad2-F (GA908) Q4275

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81  GACACCGCCCTTCACTGTCGGAGAACTCAAGAAAGCAATC Fad2-F wt
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Fig. 2A

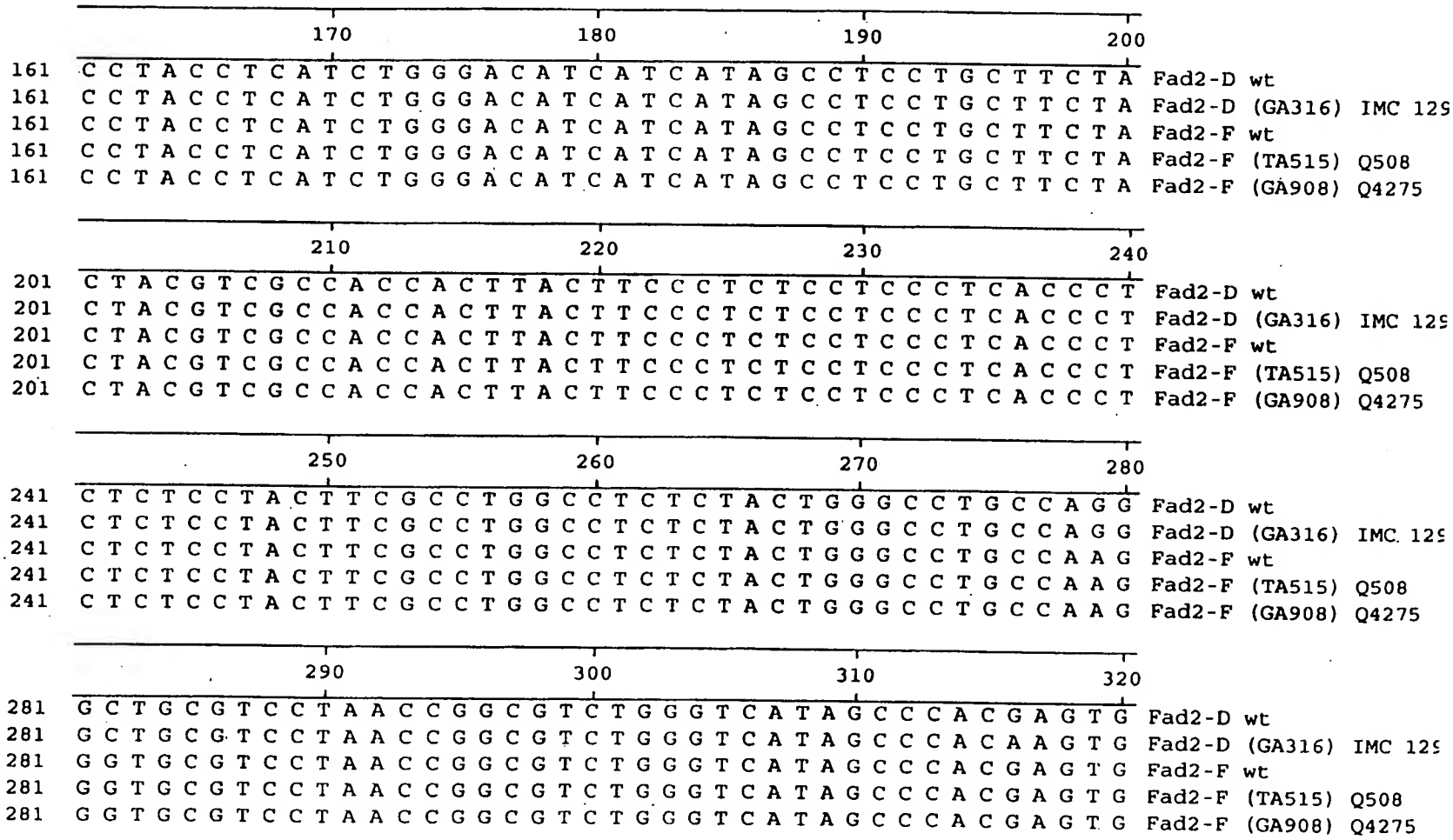
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Fig. 2B

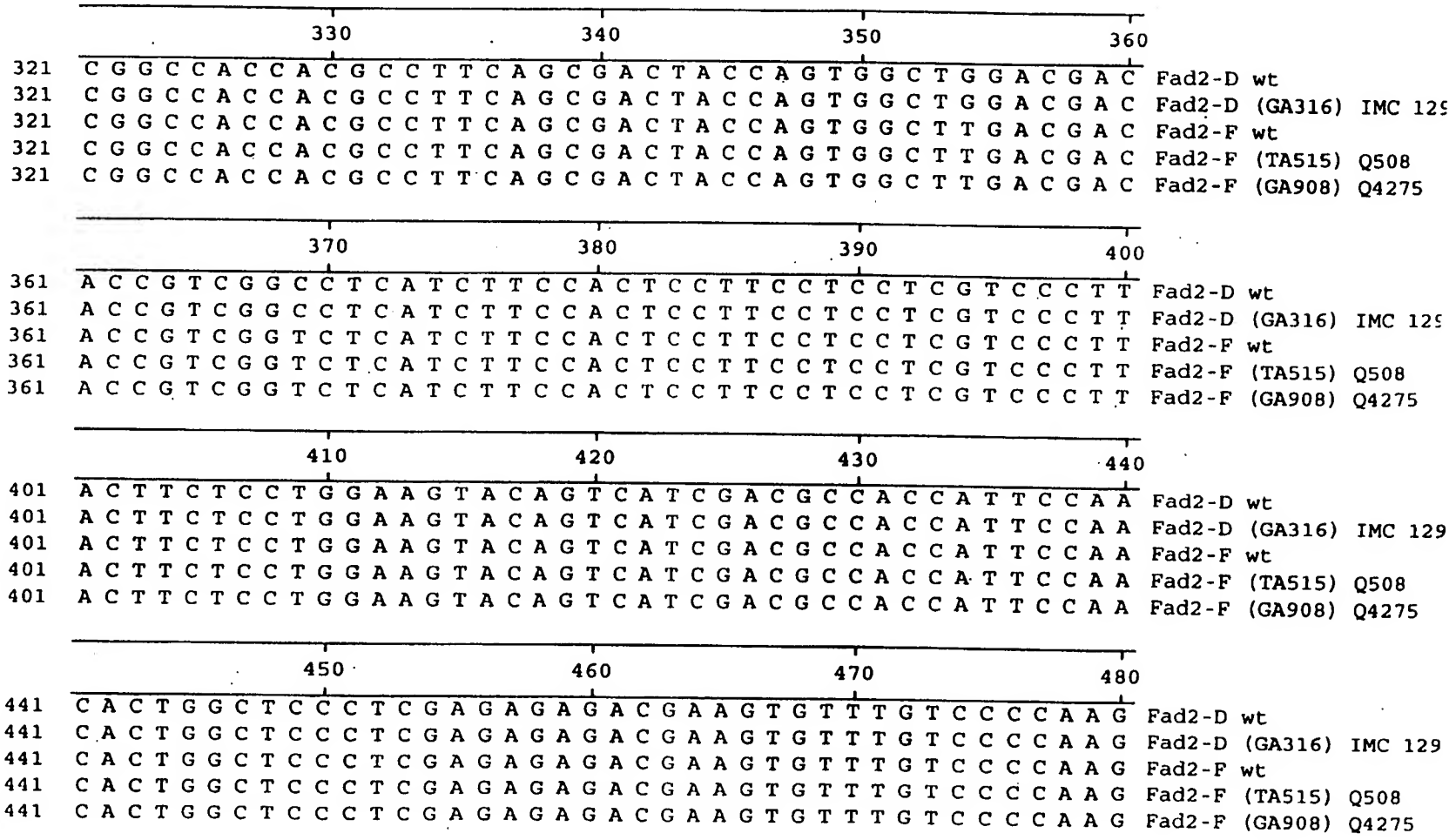
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Fig. 2C

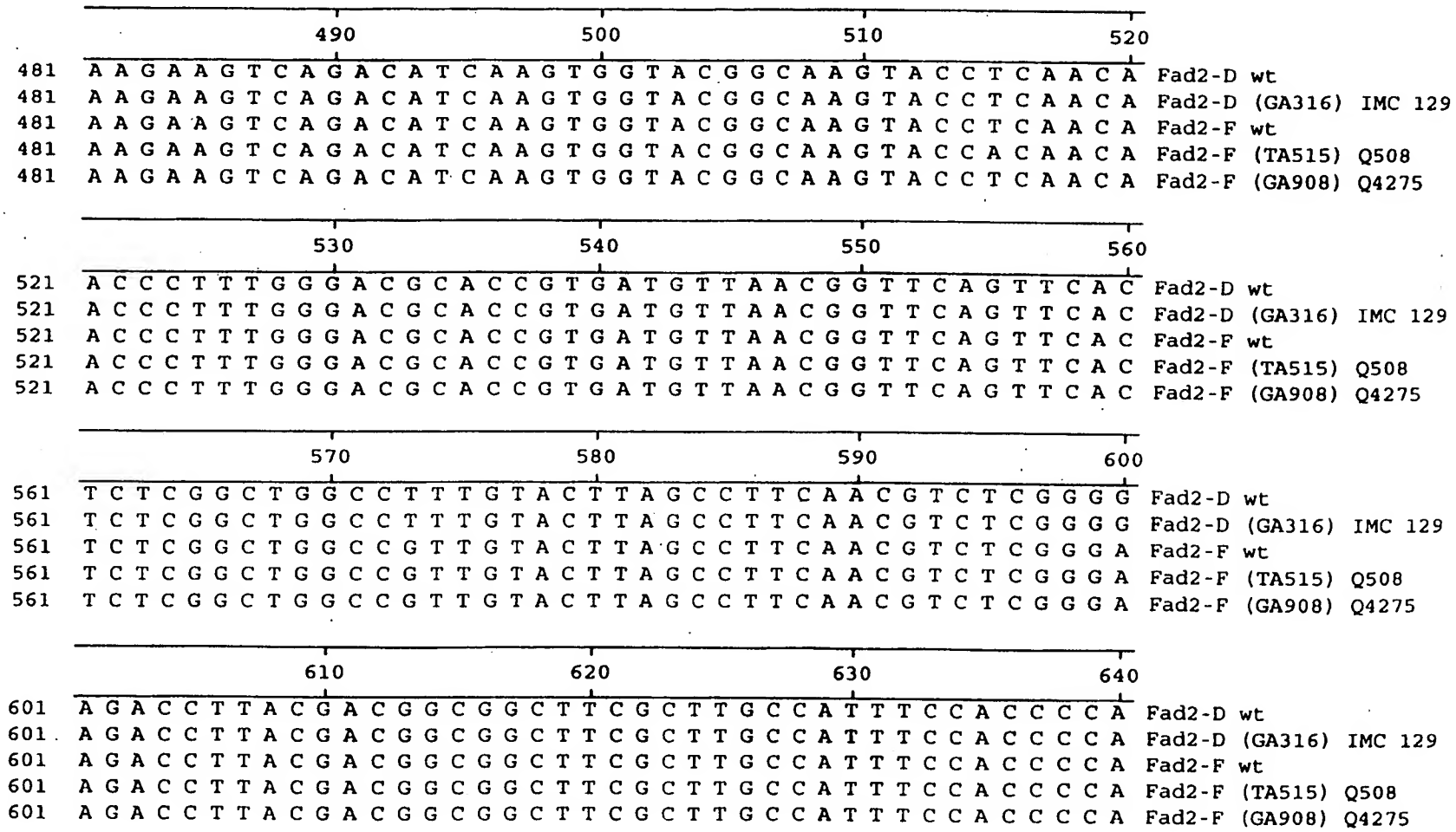
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Fig. 2D

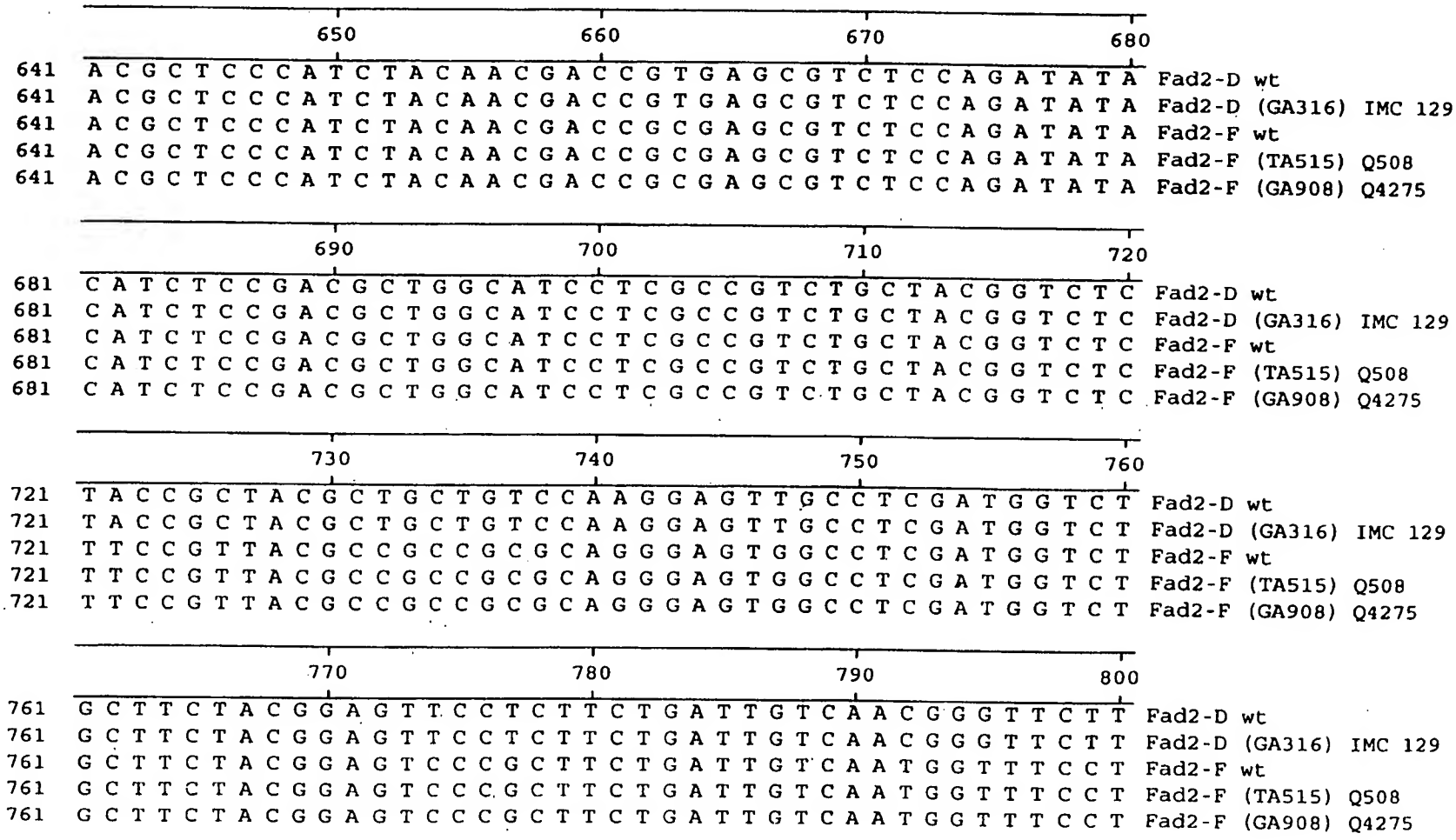
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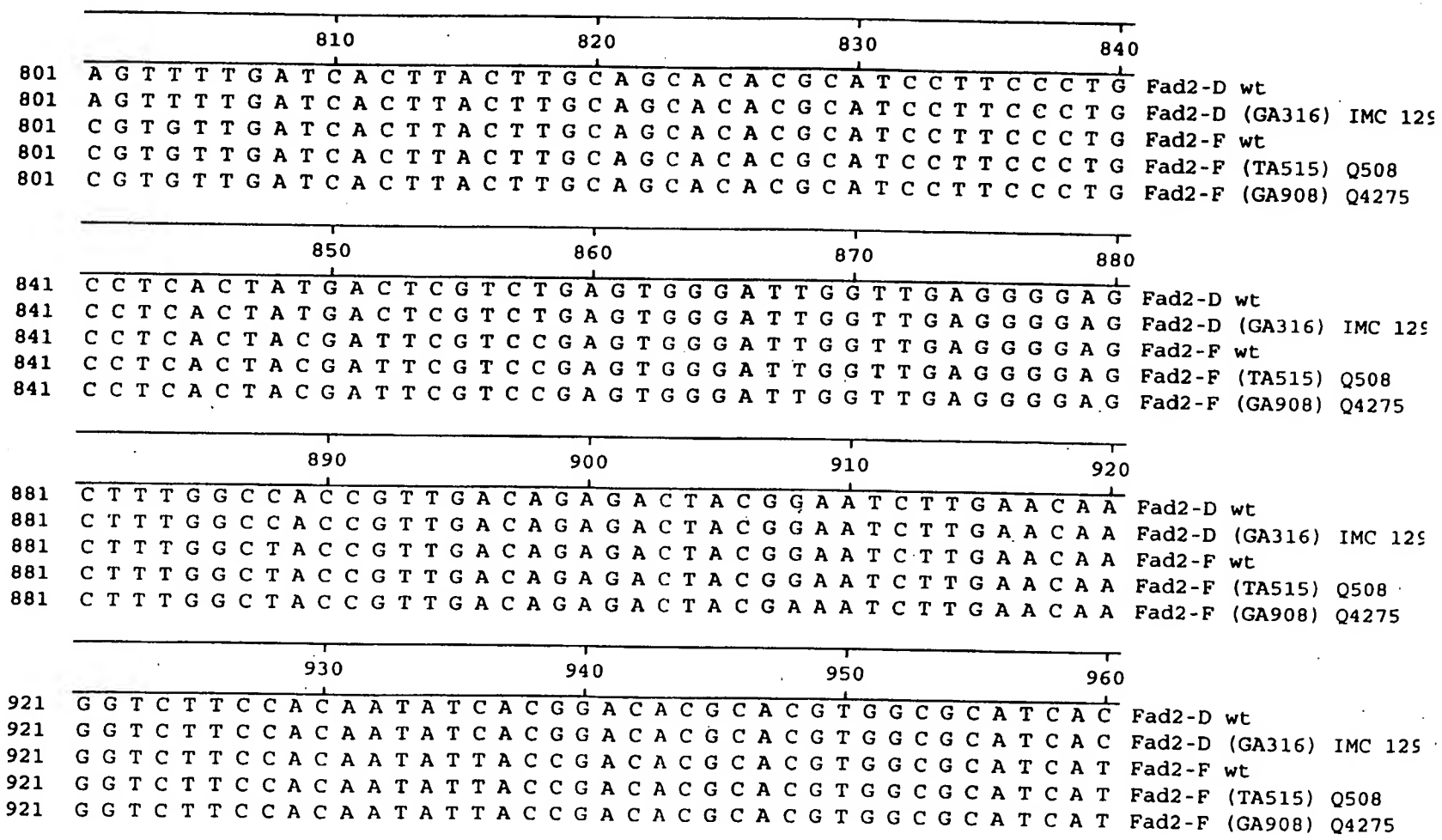
Fig. 2E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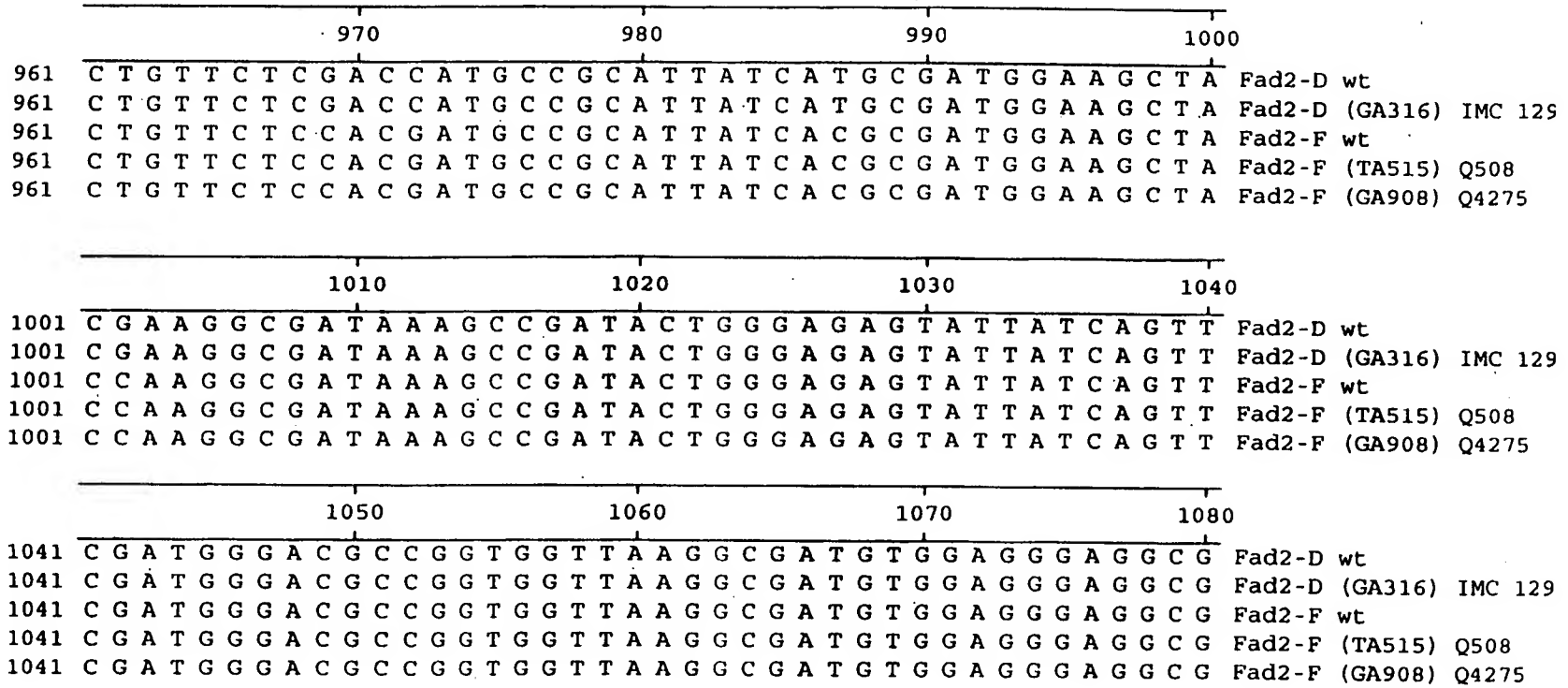
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1001 CCAAGGCGATAAAGCCGATACTGGGAGAGTATTATCAGTT Fad2-F (GA908) Q4275
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Fig. 2G

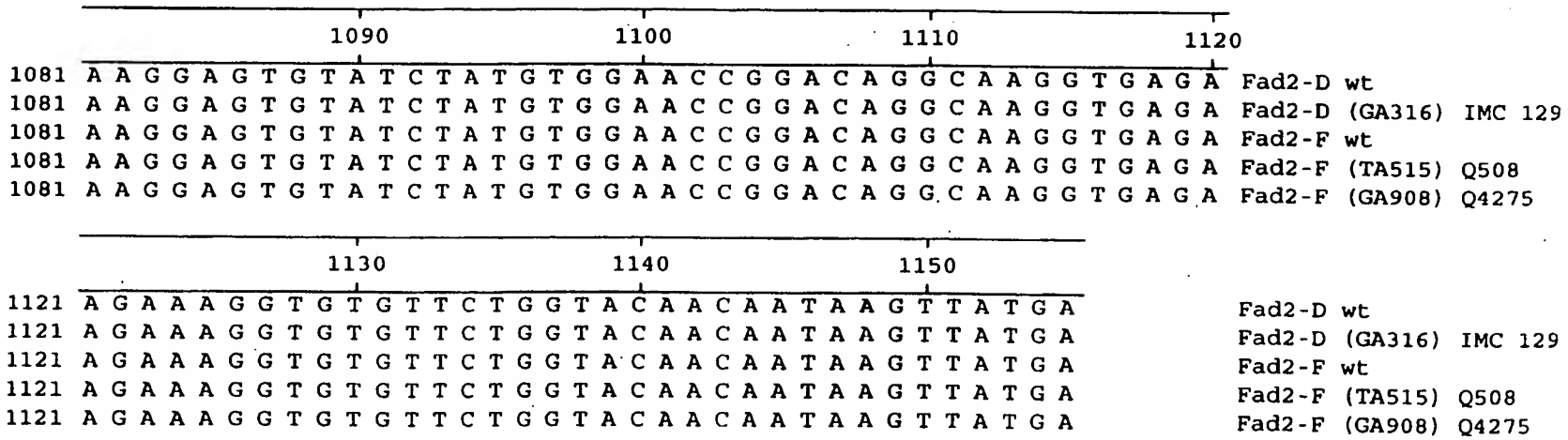
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Fig. 2H

```
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1   Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser Glu Thr Asp Asn  Fad2-D (GA316) IMC129
1   Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser Glu Thr Asp Thr  Fad2-F wt
1   Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser Glu Thr Asp Thr  Fad2-F (TA515) Q508
1   Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser Glu Thr Asp Thr  Fad2-F (GA908) Q4275

                                  30                  40
21  Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr Val Gly Glu Leu Lys Lys Ala Ile  Fad2-D wt
21  Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr Val Gly Glu Leu Lys Lys Ala Ile  Fad2-D (GA316) IMC129
21  Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr Val Gly Glu Leu Lys Lys Ala Ile  Fad2-F wt
21  Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr Val Gly Glu Leu Lys Lys Ala Ile  Fad2-F (TA515) Q508
21  Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr Val Gly Glu Leu Lys Lys Ala Ile  Fad2-F (GA908) Q4275

                                  50                  60
41  Pro Pro His Cys Phe Lys Arg Ser Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile  Fad2-D wt
41  Pro Pro His Cys Phe Lys Arg Ser Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile  Fad2-D (GA316) IMC129
41  Pro Pro His Cys Phe Lys Arg Ser Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile  Fad2-F wt
41  Pro Pro His Cys Phe Lys Arg Ser Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile  Fad2-F (TA515) Q508
41  Pro Pro His Cys Phe Lys Arg Ser Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile  Fad2-F (GA908) Q4275

                                  70                  80
61  Ile Ile Ala Ser Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  Fad2-D wt
61  Ile Ile Ala Ser Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  Fad2-D (GA316) IMC129
61  Ile Ile Ala Ser Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  Fad2-F wt
61  Ile Ile Ala Ser Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  Fad2-F (TA515) Q508
61  Ile Ile Ala Ser Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  Fad2-F (GA908) Q4275
```

Fig. 3A

```
                                        90                  100
81  Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val Leu Thr Gly Val  Fad2-D wt
81  Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val Leu Thr Gly Val  Fad2-D (GA316) IMC129
81  Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val Leu Thr Gly Val  Fad2-F wt
81  Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val Leu Thr Gly Val  Fad2-F (TA515) Q508
81  Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val Leu Thr Gly Val  Fad2-F (GA908) Q4275

                                       110                  120
101 Trp Val Ile Ala His Glu Cys Gly His His Ala Phe Ser Asp Tyr Gln Trp Leu Asp Asp  Fad2-D wt
101 Trp Val Ile Ala His Lys Cys Gly His His Ala Phe Ser Asp Tyr Gln Trp Leu Asp Asp  Fad2-D (GA316) IMC129
101 Trp Val Ile Ala His Glu Cys Gly His His Ala Phe Ser Asp Tyr Gln Trp Leu Asp Asp  Fad2-F wt
101 Trp Val Ile Ala His Glu Cys Gly His His Ala Phe Ser Asp Tyr Gln Trp Leu Asp Asp  Fad2-F (TA515) Q508
101 Trp Val Ile Ala His Glu Cys Gly His His Ala Phe Ser Asp Tyr Gln Trp Leu Asp Asp  Fad2-F (GA908) Q4275

                                       130                  140
121 Thr Val Gly Leu Ile Phe His Ser Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser  Fad2-D wt
121 Thr Val Gly Leu Ile Phe His Ser Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser  Fad2-D (GA316) IMC129
121 Thr Val Gly Leu Ile Phe His Ser Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser  Fad2-F wt
121 Thr Val Gly Leu Ile Phe His Ser Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser  Fad2-F (TA515) Q508
121 Thr Val Gly Leu Ile Phe His Ser Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser  Fad2-F (GA908) Q4275

                                       150                  160
141 His Arg Arg His His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  Fad2-D wt
141 His Arg Arg His His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  Fad2-D (GA316) IMC129
141 His Arg Arg His His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  Fad2-F wt
141 His Arg Arg His His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  Fad2-F (TA515) Q508
141 His Arg Arg His His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  Fad2-F (GA908) Q4275
```

Fig. 3B

```
                                        170                                 180
161 Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu Gly Arg Thr Val  Fad2-D wt
161 Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu Gly Arg Thr Val  Fad2-D (GA316) IMC129
161 Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu Gly Arg Thr Val  Fad2-F wt
161 Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr His Asn Asn Pro Leu Gly Arg Thr Val  Fad2-F (TA515) Q508
161 Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu Gly Arg Thr Val  Fad2-F (GA908) Q4275

                                        190                                 200
181 Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu Tyr Leu Ala Phe Asn Val Ser Gly  Fad2-D wt
181 Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu Tyr Leu Ala Phe Asn Val Ser Gly  Fad2-D (GA316) IMC129
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181 Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu Tyr Leu Ala Phe Asn Val Ser Gly  Fad2-F (TA515) Q508
181 Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu Tyr Leu Ala Phe Asn Val Ser Gly  Fad2-F (GA908) Q4275

                                        210                                 220
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201 Arg Pro Tyr Asp Gly Gly Phe Ala Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp  Fad2-D (GA316) IMC129
201 Arg Pro Tyr Asp Gly Gly Phe Ala Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp  Fad2-F wt
201 Arg Pro Tyr Asp Gly Gly Phe Ala Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp  Fad2-F (TA515) Q508
201 Arg Pro Tyr Asp Gly Gly Phe Ala Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp  Fad2-F (GA908) Q4275

                                        230                                 240
221 Arg Glu Arg Leu Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  Fad2-D wt
221 Arg Glu Arg Leu Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  Fad2-D (GA316) IMC129
221 Arg Glu Arg Leu Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  Fad2-F wt
221 Arg Glu Arg Leu Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  Fad2-F (TA515) Q508
221 Arg Glu Arg Leu Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  Fad2-F (GA908) Q4275
```

Fig. 3C

```
                                  250                                 260
241 Tyr Arg Tyr Ala Ala Val Gln Gly Val Ala Ser Met Val Cys Phe Tyr Gly Val Pro Leu  Fad2-D wt
241 Tyr Arg Tyr Ala Ala Val Gln Gly Val Ala Ser Met Val Cys Phe Tyr Gly Val Pro Leu  Fad2-D (GA316) IMC129
241 Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr Gly Val Pro Leu  Fad2-F wt
241 Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr Gly Val Pro Leu  Fad2-F (TA515) Q508
241 Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr Gly Val Pro Leu  Fad2-F (GA908) Q4275

                                  270                                 280
261 Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr Leu Gln His Thr His Pro Ser Leu  Fad2-D wt
261 Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr Leu Gln His Thr His Pro Ser Leu  Fad2-D (GA316) IMC129
261 Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr Leu Gln His Thr His Pro Ser Leu  Fad2-F wt
261 Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr Leu Gln His Thr His Pro Ser Leu  Fad2-F (TA515) Q508
261 Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr Leu Gln His Thr His Pro Ser Leu  Fad2-F (GA908) Q4275

                                  290                                 300
281 Pro His Tyr Asp Ser Ser Glu Trp Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg  Fad2-D wt
281 Pro His Tyr Asp Ser Ser Glu Trp Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg  Fad2-D (GA316) IMC129
281 Pro His Tyr Asp Ser Ser Glu Trp Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg  Fad2-F wt
281 Pro His Tyr Asp Ser Ser Glu Trp Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg  Fad2-F (TA515) Q508
281 Pro His Tyr Asp Ser Ser Glu Trp Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg  Fad2-F (GA908) Q4275

                                  310                                 320
301 Asp Tyr Gly Ile Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  Fad2-D wt
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301 Asp Tyr Gly Ile Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  Fad2-F wt
301 Asp Tyr Gly Ile Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  Fad2-F (TA515) Q508
301 Asp Tyr Glu Ile Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  Fad2-F (GA908) Q4275
```

Fig. 3D

```
                                        330                 340
321 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala Ile Lys Pro Ile  Fad2-D wt
321 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala Ile Lys Pro Ile  Fad2-D (GA316) IMC129
321 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala Ile Lys Pro Ile  Fad2-F wt
321 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala Ile Lys Pro Ile  Fad2-F (TA515) Q508
321 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala Ile Lys Pro Ile  Fad2-F (GA908) Q4275

                                        350                 360
341 Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val Val Lys Ala Met Trp Arg Glu Ala  Fad2-D wt
341 Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val Val Lys Ala Met Trp Arg Glu Ala  Fad2-D (GA316) IMC129
341 Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val Val Lys Ala Met Trp Arg Glu Ala  Fad2-F wt
341 Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val Val Lys Ala Met Trp Arg Glu Ala  Fad2-F (TA515) Q508
341 Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val Val Lys Ala Met Trp Arg Glu Ala  Fad2-F (GA908) Q4275

                                        370                 380
361 Lys Glu Cys Ile Tyr Val Glu Pro Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr  Fad2-D wt
361 Lys Glu Cys Ile Tyr Val Glu Pro Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr  Fad2-D (GA316) IMC129
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361 Lys Glu Cys Ile Tyr Val Glu Pro Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr  Fad2-F (GA908) Q4275

381 Asn Asn Lys Leu ter                                                              Fad2-D wt
381 Asn Asn Lys Leu ter                                                              Fad2-D (GA316) IMC129
381 Asn Asn Lys Leu ter                                                              Fad2-F wt
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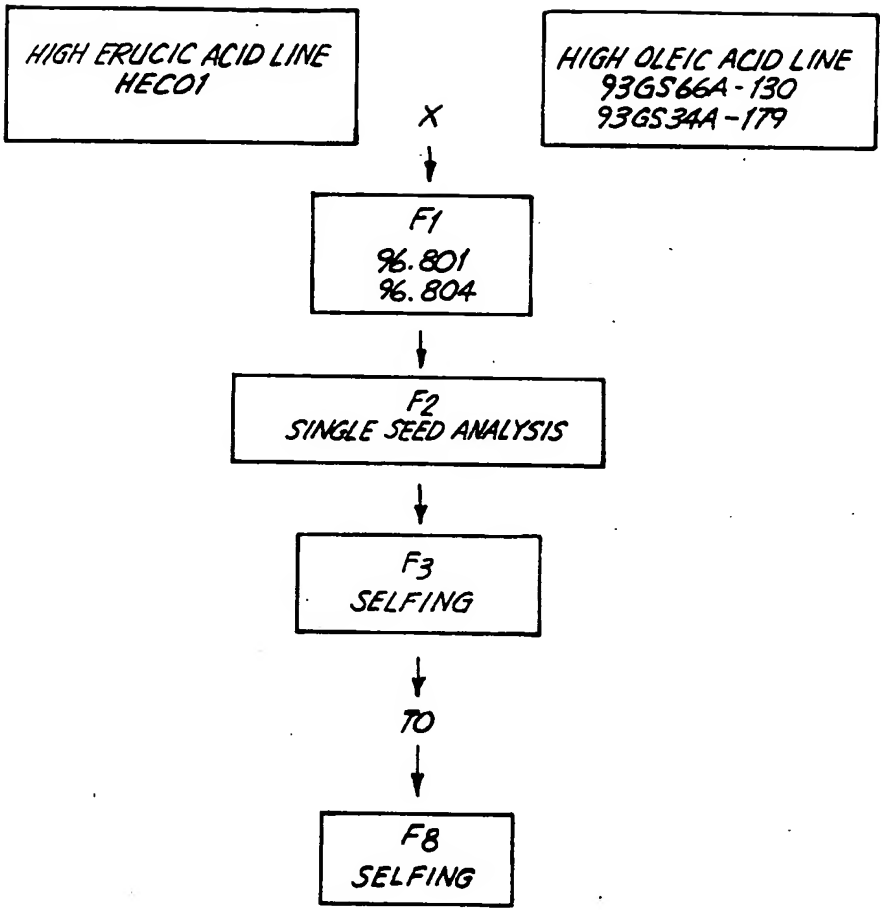
Fig. 3E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TO
F8
SELFING

Fig. 4

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DeBonte, Lorin R.

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tga 1155

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<211> 384
<212> PRT
<213> Brassica napus

<220>
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<222> (133)...(133)
<223> Xaa = Pro or Leu

<221> UNSURE
<222> (194)...(194)
<223> Xaa = Leu

<221> UNSURE
<222> (246)...(246)
<223> Xaa = Ile or Val

<221> UNSURE
<222> (262)...(262)
<223> Xaa = Ile or Val

<221> UNSURE
<222> (345)...(345)
<223> Xaa = Tyr

<400> 2

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Glu Thr Asp Asn Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
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Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
35 40 45
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
50 55 60
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65 70 75 80
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
85 90 95
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
100 105 110
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
115 120 125
Phe Leu Leu Val Xaa Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His

130 135 140
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145 150 155 160
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
165 170 175
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
180 185 190
Tyr Xaa Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
195 200 205
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
210 215 220
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225 230 235 240
Tyr Arg Tyr Ala Ala Xaa Gln Gly Val Ala Ser Met Val Cys Phe Tyr
245 250 255
Gly Val Pro Leu Leu Xaa Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
260 265 270
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
275 280 285
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
290 295 300
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305 310 315 320
Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
325 330 335
Ile Lys Pro Ile Leu Gly Glu Tyr Xaa Gln Phe Asp Gly Thr Pro Val
340 345 350
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
355 360 365
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
370 375 380

<210> 3
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<223> Xaa = Leu

<221> unsure
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<223> Xaa = Ile or Val

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<221> unsure
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<223> Xaa = Tyr

<400> 3

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 1               5                  10                  15

gaa acc gac aac atc aag cgc gta ccc tgc gag aca ccg ccc ttc act          96
Glu Thr Asp Asn Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
            20                  25                  30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg         144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
        35                  40                  45

atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc         192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    50                  55                  60

tgc ttc tac tac gtc gcc acc act tac ttc cct ctc ctc cct cac cct         240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65                  70                  75                  80

ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc cag ggc tgc gtc         288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
                85                  90                  95

cta acc ggc gtc tgg gtc ata gcc cac aag tgc ggc cac cac gcc ttc         336
Leu Thr Gly Val Trp Val Ile Ala His Lys Cys Gly His His Ala Phe
            100                 105                 110

agc gac tac cag tgg ctg gac gac acc gtc ggc ctc atc ttc cac tcc         384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
        115                 120                 125

ttc ctc ctc gtc cyt tac ttc tcc tgg aag tac agt cat cga cgc cac         432
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Phe Leu Leu Val Xaa Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
130 135 140

cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag 480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145 150 155 160

aag aag tca gac atc aag tgg tac ggc aag tac ctc aac aac cct ttg 528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
165 170 175

gga cgc acc gtg atg tta acg gtt cag ttc act ctc ggc tgg cct ttg 576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
180 185 190

tac ttr gcc ttc aac gtc tcg ggg aga cct tac gac ggc ggc ttc gct 624
Tyr Xaa Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
195 200 205

tgc cat ttc cac ccc aac gct ccc atc tac aac gac cgt gag cgt ctc 672
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
210 215 220

cag ata tac atc tcc gac gct ggc atc ctc gcc gtc tgc tac ggt ctc 720
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225 230 235 240

tac cgc tac gct gct rtc caa gga gtt gcc tcg atg gtc tgc ttc tac 768
Tyr Arg Tyr Ala Ala Xaa Gln Gly Val Ala Ser Met Val Cys Phe Tyr
245 250 255

gga gtt cct ctt ctg rtt gtc aac ggg ttc tta gtt ttg atc act tac 816
Gly Val Pro Leu Leu Xaa Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
260 265 270

ttg cag cac acg cat cct tcc ctg cct cac tat gac tcg tct gag tgg 864
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
275 280 285

gat tgg ttg agg gga gct ttg gcc acc gtt gac aga gac tac gga atc 912
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
290 295 300

ttg aac aag gtc ttc cac aat atc acg gac acg cac gtg gcg cat cac 960
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305 310 315 320

ctg ttc tcg acc atg ccg cat tat cat gcg atg gaa gct acg aag gcg 1008

Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
325 330 335

ata aag ccg ata ctg gga gag tat tay cag ttc gat ggg acg ccg gtg 1056
Ile Lys Pro Ile Leu Gly Glu Tyr Xaa Gln Phe Asp Gly Thr Pro Val
340 345 350

gtt aag gcg atg tgg agg gag gcg aag gag tgt atc tat gtg gaa ccg 1104
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
355 360 365

gac agg caa ggt gag aag aaa ggt gtg ttc tgg tac aac aat aag tta 1152
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
370 375 380

tga 1155

<210> 4
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<220>
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<223> Xaa = Ile or Val

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<223> Xaa = Tyr

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Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
35 40 45
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
50 55 60
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65 70 75 80
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
85 90 95
Leu Thr Gly Val Trp Val Ile Ala His Lys Cys Gly His His Ala Phe
100 105 110
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
115 120 125
Phe Leu Leu Val Xaa Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
130 135 140
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145 150 155 160
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
165 170 175
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
180 185 190
Tyr Xaa Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
195 200 205
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
210 215 220
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225 230 235 240
Tyr Arg Tyr Ala Ala Xaa Gln Gly Val Ala Ser Met Val Cys Phe Tyr
245 250 255
Gly Val Pro Leu Leu Xaa Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
260 265 270
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
275 280 285
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
290 295 300
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305 310 315 320
Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
325 330 335
Ile Lys Pro Ile Leu Gly Glu Tyr Xaa Gln Phe Asp Gly Thr Pro Val
340 345 350
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
355 360 365
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
370 375 380

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<220>
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<222> (1)...(1152)

<400> 5

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Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser
 1               5                  10                  15

gaa acc gac acc atc aag cgc gta ccc tgc gag aca ccg ccc ttc act          96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
            20                  25                  30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg         144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
        35                  40                  45

atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc         192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    50                  55                  60

tgc ttc tac tac gtc gcc acc act tac ttc cct ctc ctc cct cac cct         240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65                  70                  75                  80

ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc caa ggg tgc gtc         288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
                85                  90                  95

cta acc ggc gtc tgg gtc ata gcc cac gag tgc ggc cac cac gcc ttc         336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
            100                 105                 110

agc gac tac cag tgg ctt gac gac acc gtc ggt ctc atc ttc cac tcc         384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
        115                 120                 125

ttc ctc ctc gtc cct tac ttc tcc tgg aag tac agt cat cga cgc cac         432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
    130                 135                 140

cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag         480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145                 150                 155                 160
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aag aag tca gac atc aag tgg tac ggc aag tac ctc aac aac cct ttg 528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
165 170 175

gga cgc acc gtg atg tta acg gtt cag ttc act ctc ggc tgg ccg ttg 576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
180 185 190

tac tta gcc ttc aac gtc tcg gga aga cct tac gac ggc ggc ttc gct 624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
195 200 205

tgc cat ttc cac ccc aac gct ccc atc tac aac gac cgc gag cgt ctc 672
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
210 215 220

cag ata tac atc tcc gac gct ggc atc ctc gcc gtc tgc tac ggt ctc 720
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225 230 235 240

ttc cgt tac gcc gcc gcg cag gga gtg gcc tcg atg gtc tgc ttc tac 768
Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr
245 250 255

gga gtc ccg ctt ctg att gtc aat ggt ttc ctc gtg ttg atc act tac 816
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
260 265 270

ttg cag cac acg cat cct tcc ctg cct cac tac gat tcg tcc gag tgg 864
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
275 280 285

gat tgg ttg agg gga gct ttg gct acc gtt gac aga gac tac gga atc 912
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
290 295 300

ttg aac aag gtc ttc cac aat att acc gac acg cac gtg gcg cat cat 960
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305 310 315 320

ctg ttc tcc acg atg ccg cat tat cac gcg atg gaa gct acc aag gcg 1008
Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
325 330 335

ata aag ccg ata ctg gga gag tat tat cag ttc gat ggg acg ccg gtg 1056
Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
340 345 350

gtt aag gcg atg tgg agg gag gcg aag gag tgt atc tat gtg gaa ccg 1104
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
355 360 365

gac agg caa ggt gag aag aaa ggt gtg ttc tgg tac aac aat aag tta 1152
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
370 375 380

tga 1155

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<213> Brassica napus

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Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
35 40 45
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
50 55 60
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65 70 75 80
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
85 90 95
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
100 105 110
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
115 120 125
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
130 135 140
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145 150 155 160
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
165 170 175
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
180 185 190
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
195 200 205
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
210 215 220
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225 230 235 240
Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr
245 250 255

Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
260 265 270
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
275 280 285
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
290 295 300
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305 310 315 320
Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
325 330 335
Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
340 345 350
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
355 360 365
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
370 375 380

<210> 7
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<213> Brassica napus

<220>
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<222> (1)...(1152)

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gaa acc gac acc atc aag cgc gta ccc tgc gag aca ccg ccc ttc act 96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
20 25 30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg 144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
35 40 45

atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc 192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
50 55 60

tgc ttc tac tac gtc gcc acc act tac ttc cct ctc ctc cct cac cct 240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65 70 75 80

ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc caa ggg tgc gtc 288

Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
85 90 95

cta acc ggc gtc tgg gtc ata gcc cac gag tgc ggc cac cac gcc ttc 336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
100 105 110

agc gac tac cag tgg ctt gac gac acc gtc ggt ctc atc ttc cac tcc 384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
115 120 125

ttc ctc ctc gtc cct tac ttc tcc tgg aag tac agt cat cga cgc cac 432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
130 135 140

cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag 480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145 150 155 160

aag aag tca gac atc aag tgg tac ggc aag tac cac aac aac cct ttg 528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr His Asn Asn Pro Leu
165 170 175

gga cgc acc gtg atg tta acg gtt cag ttc act ctc ggc tgg ccg ttg 576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
180 185 190

tac tta gcc ttc aac gtc tcg gga aga cct tac gac ggc ggc ttc gct 624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
195 200 205

tgc cat ttc cac ccc aac gct ccc atc tac aac gac cgc gag cgt ctc 672
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
210 215 220

cag ata tac atc tcc gac gct ggc atc ctc gcc gtc tgc tac ggt ctc 720
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225 230 235 240

ttc cgt tac gcc gcc gcg cag gga gtg gcc tcg atg gtc tgc ttc tac 768
Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr
245 250 255

gga gtc ccg ctt ctg att gtc aat ggt ttc ctc gtg ttg atc act tac 816
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
260 265 270

ttg cag cac acg cat cct tcc ctg cct cac tac gat tcg tcc gag tgg 864

Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
275 280 285

gat tgg ttg agg gga gct ttg gct acc gtt gac aga gac tac gga atc 912
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
290 295 300

ttg aac aag gtc ttc cac aat att acc gac acg cac gtg gcg cat cat 960
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305 310 315 320

ctg ttc tcc acg atg ccg cat tat cac gcg atg gaa gct acc aag gcg 1008
Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
325 330 335

ata aag ccg ata ctg gga gag tat tat cag ttc gat ggg acg ccg gtg 1056
Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
340 345 350

gtt aag gcg atg tgg agg gag gcg aag gag tgt atc tat gtg gaa ccg 1104
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
355 360 365

gac agg caa ggt gag aag aaa ggt gtg ttc tgg tac aac aat aag tta 1152
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
370 375 380

tga 1155

<210> 8
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<213> Brassica napus

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Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
35 40 45
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
50 55 60
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
65 70 75 80
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
85 90 95
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe

100 105 110
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
115 120 125
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His
130 135 140
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145 150 155 160
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr His Asn Asn Pro Leu
165 170 175
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
180 185 190
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala
195 200 205
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
210 215 220
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225 230 235 240
Phe Arg Tyr Ala Ala Ala Gln Gly Val Ala Ser Met Val Cys Phe Tyr
245 250 255
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
260 265 270
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
275 280 285
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
290 295 300
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305 310 315 320
Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
325 330 335
Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
340 345 350
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
355 360 365
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
370 375 380

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<222> (1)...(1152)

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gaa acc gac acc atc aag cgc gta ccc tgc gag aca ccg ccc ttc act 96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
20 25 30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg 144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17645

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01H 5/00, 1/02; C09K 7/02; C12P 7/40, 7/64; C12N 9/20
US CL :800/264, 298; 426/601; 305/117
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/264, 298; 426/601; 305/117

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST/DERWENT, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TOPFER et al. Modification of Plant Lipid Synthesis. Science. 05 May 1995, Vol. 268, pages 681-686, see entire document.	1-38
Y	AXTELL, J.D. Breeding for Improved Nutritional Quality. In: Plant Breeding II (Frey, ed.) 1981, pages 365-415, see especially pages 389-395.	1-38
Y	CARR, R. Processing of Oilseed Crops. In: Oil Crops of the World (Robbelen et al, eds). 1989, pages 226-259, see especially pages 253 and 255.	16-28, 30-38
Y	US 5,703,022 A (FLOYD) 30 December 1997, col. 1-2.	16-28, 30-38

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 OCTOBER 1999	24 NOV 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ELIZABETH F. MCELWAIN Telephone No. 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17645

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,773,391 A (LAWATE et al) 30 June 1998, col. 26-35	16-28, 30-38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17645

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17645

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-15 and 29, drawn to a Brassica plant, progeny and a method of breedin brassica plants.
Group II, claim(s) 16-28, drawn to oil.
Group III, claim(s) 30-32, drawn to a method of making vegetable oil.
Group IV, claim(s) 33-35, drawn to a lubricant.
Group V, claim(s) 36-38, drawn to hydraulic fluid.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:. The inventions of groups I, II, IV and V are distinct products that differ chemically, structurally and functionally, and can be made separately and used independently, and lack a corresponding special technical feature, wherein the products of groups II, IV and V can be made by alternate methods than be extraction from the plants of group I, such as by chemical synthesis, and the plants may be used in a method other than for extraction of oil, such as for vegetable production. In addition, the invention of group III is drawn to a method of making oil, and the products of groups II, IV and V may be made by alternate methods, such as by chemical synthesis, and the plants of group I can be used for different methods, such as for vegetable production. Thus the claimed inventions lack a corresponding technical feature and the claimed products and methods lack unity.